EPA Regional Priority AFO Science Question Synthesis Document

Pharmaceuticals and Pathogens

Workshop Review Draft:

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SECTION 4: PHARMACEUTICALS AND PATHOGENS

4.1 Veterinary Pharmaceuticals and Microorganisms

Animal production in the U.S. has changed significantly over the past 30 years. With larger animal farms producing the majority of meat, dairy, and poultry, new concerns have emerged. As animal feeding operations (AFOs) house more animals, larger amounts of manure are produced. Current waste handling techniques offer little or no treatment to manure before it is released into the environment (USEPA, 2004). As a result, pathogens, antibiotics, and endocrine disrupting chemicals (EDCs) are being found in the environment. Although research about pharmaceuticals and pathogens continues to emerge, they are of great concern because they have been linked to adverse human health effects.

4.1.1 Adverse Human Health Effects of Drugs and Pathogens - Summary of Pathogens, Antibiotics, and EDCs Based on EPA's Risk Assessment (USEPA, 2004)

Pharmaceuticals and pathogens from AFOs may present a significant risk to human health. Pathogens may cause serious illness and even death in humans, the use of antibiotics creates antibiotic resistant organisms that may inhibit the effectiveness of human medications, and EDCs may interfere with human reproductive function. These materials generally enter the environment in livestock manure and urine that has runoff from land application or confinement areas. Based on the concentration of livestock and the management of manure at a facility, the amount of pathogens, antibiotics, and EDCs in surrounding areas may have the potential to cause negative impacts on human health.

4.1.1.1 Pathogens

Pathogens are biological agents that can cause disease. Although found naturally, the microorganisms present in livestock manure present the greatest source of pathogenic contamination. This waste has the potential to enter air, surface water, or groundwater sources if not properly managed. For example, runoff from land application may carry significant concentrations of microorganisms into surface water used for recreation, thereby exposing a large number of people to potentially pathogenic organisms. Fields of fresh produce and drinking water supplies are also vulnerable to pathogenic contamination from improperly treated manure used as fertilizer. Many cases of human diseases have been associated with consumption of fresh vegetables possibly contaminated with manure from animals. Pathogens can also be spread from animal to animal at AFOs, from human to human, and from direct contact between human and production animal (USEPA, 2004; Valcour, *et al.*, 2002).

According to the Center for Disease Control (CDC), each year 38.3 million cases of acute gastroenteritis are caused by known pathogens, of which, 13.6 million (36%) can be attributed to foodborne transmission. Of the 13.6 million cases of foodborne illnesses, 30% are caused by bacteria, 3% by parasites, and 67% by viruses (Mead, *et al.*, 1999). USDA's Economic Research Service (ERS) estimates that in the U.S., pathogens contribute to $6.9 billion in medical costs, lost productivity, and premature deaths each year (Buzby, 2001). Table 4.1

provides a list of pathogens common to farms, the corresponding human disease, and the animal source for the pathogens.

Table 4-1. Diseases from Pathogens Found in Animal Wastes and on Farms (Buzby, 2001; Hill, 1999; Mead, *et al.*, 1999 USEPA, 2004; and http://www.epa.gov/agriculture/ag101/impactpathogens.html).

Pathogen	Human Disease	Animal Source
Campylobacter	Campylobacteriosis; Guillain-Barre syndrome; reactive arthritis	Swine, poultry, and cattle
Cryptosporidium parvum	Cryptosporidiosis	Cattle
Escherichia coli O157:H7	Hemorrhagic colitis; Hemolytic uremic syndrome (HUS); Enterohaemorrhagic colibacillosis	Cattle
Giardia	Giardiasis	Cattle
Listeria monocytogenes	Listeriosis	Cattle, ruminants
Salmonella	Salmonellosis	Swine, poultry, and cattle
Yersinia enterocolitica	Yersiniosis	Pigs

4.1.1.2 Antibiotics

Pharmaceutical use in animal production also poses a threat to humans. Antibiotics are beneficial and used extensively in animal production to treat infections, promote growth, and use in prophylatic care. However, there are concerns about the broader effects of widespread antibiotic use in animal operations. One of the primary concerns is the increase in the populations of drug-resistant microbes in the environment. Antibiotic residues may be found in animal waste and may enter surface or groundwater and come into contact with humans.

Microorganisms may build up an antibiotic resistance and become unresponsive to the antibiotic used to treat them. This scenario leads to bacterial infections for which there is no effective antibiotic. In these cases, humans would be susceptible to illness and possibly death from resistant strains of bacterium (USEPA, 2004).

4.1.1.3 Endocrine Disrupting Chemicals

EPA has defined an endocrine disrupting chemical (EDC) as “an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior” (USEPA, 2004). EDCs may result in a variety of unhealthy

outcomes. For example, an EDC may initiate a health-related outcome in humans or wildlife by binding to and stimulating estrogen and androgen receptors. Steroid hormones are chemicals of concerns to endocrine health associated with AFOs. Animals remove hormones from their bodies by excreting them in urine or feces. As with pathogens and antibiotics, EDCs may enter surface or groundwater and come into contact with humans. The classes of natural (biogenic) hormones that may be excreted by animals include estrogens, androgens, progesterones, and thyroid hormones.

A variety of chemicals, including certain pesticides have been found to cause endocrine disruption in laboratory animal studies. Observed effects included disruption of female and male reproductive function (such as disruption of normal sexual differentiation, ovarian function, sperm production, and pregnancy) (Orlando, *et al.*, 2001) and effects on the thyroid gland (which helps maintain normal metabolism). However, a relationship between exposure to a specific environmental agent and an adverse health effect in humans operating via endocrine disruption has not been established (USEPA, 1997). More information is needed about potential health effects of EDCs.

4.1.2 Analytical Methods Available for Use in an Environmental Setting

Based on the uncertainty of health affects from pathogens, antibiotics, and EDCs, analytical methods can be used to understand their impacts on humans and the environment. Laboratory analytical methods are used by industries and municipalities to analyze the chemical and biological components of wastewater, drinking water, sediment, and other environmental samples that are required by regulations under the authority of the Clean Water Act (CWA) and the Safe Drinking Water Act (SDWA). This testing supplies information about the amount of pathogens, antibiotics, and EDCs making their way into our environment and potentially into our drinking water.

4.1.2.1 Analytical Methods for Pathogens

Epidemiological studies suggest a positive relationship between high concentrations of the bacterium *E. coli* and enterococci in ambient waters and incidents of gastrointestinal illnesses associated with swimming. Several studies support the use of *E. coli* and enterococci (instead of fecal coliform) as indicators of microbiological pollution. Studies also show that *Cryptosporidium* and *Giardia* can cause gastrointestinal illness when they are present in ambient waters. The following analytical methods will help the scientific community better assess public health risks from microbiological pollutants in ambient waters. The following section describes research for the pathogens: protozoa (*Cryptosporidium* and *Giardia*); bacteria (*E. Coli*, enterococci, and *Campylobacter*, *Arcobacter*, and *Helicobacter*); and viruses.

4.1.2.1.1 Protozoa

Cryptosporidium and *Giardia*

Several analytical methods have been developed for detecting protozoa (*Cryptosporidium* and *Giardia*) in water and fecal samples. These methods include: EPA Method 1622, EPA Method 1623, and immunofluorescence.

EPA has developed two methods for detecting *Cryptosporidium* in water. EPA Method 1622 was validated for use as a test solely for *Cryptosporidium*. EPA Method 1622 is a performance-based method applicable to the determination of *Cryptosporidium* in aqueous matrices (USEPA, 2001a). EPA Method 1622 requires filtration, immunomagnetic separation of the oocysts from the material captured, and an immunofluorescence assay for determination of oocyst concentrations, with confirmation through vital dye staining and differential interference contrast microscopy (USEPA, 2001a). In 1999, EPA validated a method for simultaneous detection of *Cryptosporidium* and *Giardia* that was designated EPA Method 1623. The difference between EPA Method 1622 and EPA Method 1623 is that *Giardia* can be sampled at the same time as *Cryptosporidium*, otherwise, the two methodologies are almost identical (USEPA, 2001b).

Detecting *C. parvum* in animal feces requires slightly different analytical methods. Techniques that have been developed and optimized for enumeration of *Cryptosporidium* oocysts in water are generally not suitable for enumeration of oocysts in animal feces (Davies, 2003). The most common method for extracting *C. parvum* from fecal material is antibody-based immunofluorescence assay (Davies, 2003; CDC, 1997; Harter, *et al.*, 2000). This method involves purifying material and staining it with fluorescence antibody reagents, then examining them under the microscope. For a more accurate count of *C. parvum* oocysts, immunofluorescent microscopy uses a microscope to enumerate bacteria tagged with fluorescent antibodies. Limitations of antibody-based microscopic methods include: lack of information on the infectivity or viability of cysts or oocysts; lack of indication of the host species of origin of the organisms; chance of false identification of algal or other protzoal species as *Cryptosporidium* or *Giardia*; poor recovery efficiency; poor precision; time-consuming nature of the process; and need for an experienced and skilled microscopist (CDC, 1997). Immunofluorescence can also be used to quantify *C. parvum* oocysts in water.

In a study by das Gracas (1999), direct immunofluorescence assay (DFA) was compared to immunomagnetic separation (IMS) assay with immunofluorescent microscopy for adult bovine feces. Results found that adding IMS to DFA increased sensitivity by 2-log-unit for detecting low concentrations of oocysts of *C. parvum* in adult bovine feces. This sensitivity is attributed to IMS-DFA testing 125 times more fecal material than DFA alone. Although the cost of reagents for IMS-DFA is high, the procedure is cost-effective in comparison to DFA alone. In addition, IMS-DFA provides a relatively accurate quantification of oocysts in adult cattle feces, which is essential for developing reliable estimates for the rate of environmental loading of *C. parvum* attributable to cattle, and the risk it poses to microbial water quality at the watershed level (das Gracas, 1999).

EPA conducted a study to determine the prevalence of fecal shedding, distribution of genotypes, and associated risk factors for *C. parvum* infection in populations of feedlot cattle in the United States using IMS-DFA. Of the nine DFA+ and two IMS-DFA+ samples, four isolates had sufficient numbers of oocysts to allow molecular confirmation as *C. parvum*. Using a nested polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, the isolates were determined to be bovine genotype A. (http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/822/report/0)

Determining the genotype and subgenotype of *Cryptosporidium* helps track infections and trace contamination sources for outbreaks. Current genotyping tools differentiate *Cryptosporidium* parasites at the genotype level, which does not allow enough detail to track infections or contamination. Xiao, *et al.* (2001) discovered two double-stranded extrachromosomal virus like RNAs (ds-RNA) in *C. parvum*. This discovery allows ds-RNA to potentially act as a subgenotyping tool for *Cryptosporidium*. The extensive intragenotypic heterogeneity in the small ds-RNA sequence exhibited by isolates of the *C. parvum* bovine and human genotypes indicates that ds-RNA has potential as a high resolution tool for subgenotyping *Cryptosporidium* parasites (Xiao, *et al.*, 2001). For more information about contamination source tracking for outbreaks, see Section 4.3.

4.1.2.1.2 Bacteria

Three categories of bacteria are discussed: *E. coli*, enterococci, and *Campylobacter*, *Arcobacter*, and *Helicobacter* (collectively). EPA has developed several techniques for detecting *E. coli* and enterococci in water. In addition, the following methods are discussed: sorbitol-MacConkey agar plate, phenotypic characterization, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), and the Johnson-Murano method.

E. coli

EPA has developed three techniques to verify the presence of and the quantity of *E. coli* in water. These methods include Method 1103.1: Membrane-Thermotolerant *Escherichia coli* Agar; Method 1603: Membrane Filtering Using Modified Membrane-Thermotolerant *Escherichia coli* Agar; and Method 1604: Total Coliforms and *E. coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium). A short description of each follows:

Method 1103.1: Membrane-Thermotolerant *Escherichia coli* Agar includes filtering a water sample through a membrane. The bacteria remain on the filter and both are transferred to a selective medium (mTEC). The medium is incubated and colonies are formed. The filter is transferred to a urea saturated pad where the colonies are counted under a fluorescent light (USEPA, 2002a).

For Method 1603: Membrane Filtering Using Modified Membrane-Thermotolerant *Escherichia coli* Agar, a water sample is initially filtered through a membrane. The bacteria remain on the filter and both are transferred to a selective medium (Modified mTEC). The medium is then incubated and colonies are formed. The colonies appear red or magenta on the

modified mTEC and can be counted immediately after incubation (USEPA, 2002d). The apparatus, equipment and sampling, filtration, and verification procedure for the modified mTEC Agar method and the original mTEC Agar method are identical. The modified mTEC Agar is a single step method that uses one medium and does not require the transfer of the membrane filter to another medium or other substrate similar to Method 1103.1(USEPA, 2002d).

Method 1604: Total Coliforms and *E. coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium) uses water filtered through a pore size cellulose ester membrane. The bacteria remains on the filter and both are moved to either a MI agar or absorbent pad saturated with MI broth. The plate/pad is incubated and colonies are formed. The colonies are inspected under an ultraviolet light for the presence of a blue color, which indicates *E. coli* (USEPA, 2002e).

In addition to detecting *E. coli* in water, several studies have tested for *E. coli* in food or feces. In these instances, a slightly different methodology is suggested, the MacConkey agar plate containing sorbitol (SMAC medium) (March and Ratnam, 1986). The SMAC medium allows for ready recognition of E coli O157:H7 in stool cultures. This methodology is used by other researchers to test for *E. coli* in alfalfa seeds and sprouts (Breuer, *et al*., 2001) and in fecal samples (Cizek, *et al*., 1999; Hancock, *et al*., 1998).

Enterococci

Enterococci is a subgroup of bacteria in the fecal streptococcus group that inhabits the gastrointestinal tract of warm-blooded animals. EPA has developed two techniques to verify the presence of and quantities of *Enterococcus* in water. These methods include: Method 1106.1: Membrane Filtration Using Membrane-*Enterococcus*-Esculin Iron Agar and Method 1600: Membrane *Enterococcus* Indoxyl-β-D-Glucoside Agar. A short description of each follows:

Method 1106.1: Membrane Filtration Using Membrane-*Enterococcus*-Esculin Iron Agar filters a water sample through a membrane. The bacteria remain on the filter and both are transferred to a selective medium (mE agar). Following incubation, the filter is moved to a second medium (EIA agar) and incubated for a second time. Pink or red colonies develop and a dark precipitate is formed. The colonies are counted under a fluorescent light (USEPA, 2002b).

For Method 1600: Membrane *Enterococcus* Indoxyl-β-D-Glucoside Agar, a water sample is filtered through a membrane. The bacteria remain on the filter and both are transferred to a selective medium (mEI agar). The medium is incubated and colonies form. All colonies with a blue halo are recorded as enterococci colonies. Fluorescent light aids with visibility (USEPA, 2002c).

Campylobacter, Arcobacter,* and *Helicobacter

The analytical methods to detect *Campylobacter*, *Arcobacter*, and *Helicobacter* are: phenotypic characterization, and PCR-RFLP. Classical identification methods based on phenotypic traits are often time consuming in view of the fastidious growth characteristics of species belonging to the *Campylobacter*, *Arcobacter*, and *Helicobacter* genera (Marshall, 1999). These methods are also limited by subjective interpretive criteria, lack of standardization, and

the prevalence of biochemical atypical strains. These limitations have fueled interest in molecular approaches to identification.

A PCR-RFLP molecular identification method can differentiate many species of *Campylobacter*, *Arcobacter*, and *Helicobacter*. The PCR-RFLP method allows for rapid genetic identification of many *Campylobacter*, *Arcobacter*, and *Helicobacter* species. This method was found to be relatively simple and highly discriminatory and encompassed a broader species range in its application than previously published genotypic methods (Marshall, 1999).

Arcobacter butzleri is a pathogenic bacterium that has been found in dairy cattle, pigs, poultry, and humans. Golla, *et al.*, 2002 compared the effectiveness levels of the Johnson-Murano (JM) method (consisting of enrichment in JM broth followed by plating on JM agar) and the Collins method (consisting of enrichment in EMJH-P80 broth followed by plating on Cephalothin, Vancomycin, and Amphotericin B [CVA] agar) in the isolation of *A. butzleri.* The JM broth outperformed the EMJH-P80 broth in enriching the samples. The JM method requires only four days, compared with the 11 days required for the Collins method. The JM method is better for culturing *A. butzleri* from beef and dairy cattle (Golla, *et al.*, 2002).

4.1.2.1.3 Viruses

While bacterial contamination of water and soils and the associated health risks have been studied, attention is increasingly being focused on the hazards associated with the viral contamination of water. Viruses in sewage have been proven to contaminate water, even in the absence of indicator bacteria (USEPA, 1984). Animal production also has the ability to infect soil and water with viruses in animal waste via runoff or land application. The USEPA highlights two methods for detecting viruses in the environment. These methods include: EPA Method 1601 and EPA Method 1602. Method 1601 is used to determine the presence of somatic coliphages and Method 1602 is used to enumerate the coliphages. The USEPA Manual of Methods for Virology (USEPA, 1984) provides further information for detecting viruses.

Method 1601 (Male-specific (F+) and Somatic Coliphage in Water by Two-Step Enrichment Procedure) is designed to determine the presence or absence of male-specific somatic coliphages in ground and surface water. Coliphages are viruses (bacteriophages) that infect *E. coli* and are indicators of fecal contamination. "Method 1601 describes a qualitative (presence/absence) two-step enrichment procedure for coliphage. A 100-mL or 1-L ground water sample is supplemented with $MgCl_2$ (magnesium chloride), log-phase host bacteria (*E. coli* Famp for male-specific coliphage and *E. coli* CN-13 for somatic coliphage), with tryptic soy broth (TSB) as an enrichment step for coliphage. After an overnight incubation, samples are spotted onto a lawn of host bacteria specific for each type of coliphage, incubated, and examined for circular lysis zones, which indicate the presence of coliphages" (USEPA, 2001c). The strengths and weaknesses of this method are in Table 4-6.

Method 1602 (Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure) is a "performance-based method for enumerating male-specific (F+) and somatic coliphage in ground and surface water. This single agar layer procedure requires the addition of host bacteria, magnesium chloride, and double strength molten agar medium to the sample, followed by pouring the total volume of the mixture into plates. All plates from a single

sample are examined for plaque formation (zones of bacterial host lawn clearing). The quantity of coliphage in a sample is expressed as plaque forming units (PFU) / 100 mL." (USEPA, 2001d). The strengths and weaknesses of this method are in Table 4-6.

Bioaerosols

Bioaerosols are microorganisms or particles, gases, vapors, or fragments of biological origin (i.e., alive or released from a living organism) that are in the air. Bioaerosols can be protozoa, bacteria, viruses, or fungi) and are a vehicle for the dissemination of human and animal pathogens. The principle bioaerosol sampling methods are: impaction, impingement, gravity settling, filtration, and electrostatic precipitation. A number of culture based approaches are available to detect and characterize the specific components of bioaerosols. In impactors, agar plates containing specific or general purpose culture media are used. For impinger samples, the sample could be characterized by a suite of culture, microscopic, and molecular methods (nuleic acid, immunological, or chemical marker) (Pillai and Ricke, 2002).

4.1.2.2 Analytical Methods for Antibiotics

Effective and harmonized analytical methods for antibiotics exist for food, but not for soils (Thiele-Bruhn, 2003). Incomplete extraction of antibiotics with very polar and non-polar extractants and strong adsorption to polar and non-polar solid phase extractants (SPE) pose serious analytical problems. Thus, for the extraction of most antibiotics, the use of weakly acidic buffers in combination with organic solvents is recommended (Thiele-Bruhn, 2003). In most instances, antibiotics are separated by chromatographic techniques and then detected.

Other existing analytical methods for antibiotics in environmental samples often combine an extraction with acidic buffered solvents and liquid chromatography-mass spectrometry (LC-MS). Meyer (2000) used a modified radioimmunoassay (RIA) screening method to detect tetracycline antibiotics and a new LC-MS method to confirm the RIA results. A modified Charm II RIA test was used. The Charm II RIA test from Charm Sciences, Inc. has been approved by the Food and Drug Administration for use as a reliable screen in determining safe levels of antibiotics in milk. Data has demonstrated that the RIA procedure can be reliably used to detect samples with greater than 1 ppb of chlorotetracycline, but that for samples containing 0.5 ppb chlorotetracycline the procedure will give a false negative 50% of the time (Meyer, 2000). According to the manufacturers literature, chlorotetracycline is less reactive than tetracycline and oxytetracycline (Meyer, 2000). Thus, the detection levels established for this procedure should be a reliable screen for these other tetracycline compounds. LC-MS was used to confirm the identification of the tetracycline compounds to which the RIA tests responded. Data demonstrate that the modified RIA procedure was an effective screen for the presence of antibiotics in water and liquid waste samples. LC-MS analysis also showed that the RIA analysis was responding to chlorotetracycline and underestimated the concentration of tetracycline compounds relative to the LC-MS. The RIA is a reliable methodology for screening samples for the tetracycline class of antibiotics in water and liquid hog lagoon waste at ppb levels. Data suggests that a more sensitive method needs to be developed for analyzing water for antibiotic compounds away from the source.

Comparison of the RIA and liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) used on water and liquid manure samples has demonstrated that RIA techniques were only effective at measuring antimicrobial residues likely to contain high levels of these compounds (such as animal waste) and that LC/ESI-MS methods are required to adequately determine the presence of antimicrobial residues in samples likely to contain low levels of these compounds (Campagnolo, 2002).

4.1.2.3 Analytical Methods for EDCs

The methods to detect EDCs fall into two general categories: biological screening tools, and direct measurements (Snyder, *et al*., 2003). Biological screening tools include: in vitro bioassays and receptor binding assays. Direct measurements include: extractions, gas and liquid chromatography, and immunosorbent methods. A short description of each method follows:

Biological Screening Tools

In vitro bioassays are a quick, inexpensive, reproducible means to measure the levels of EDCs in single compounds or complex mixtures. This method measures the level of reactions caused by the activation of estrogen receptor (ER) and androgen receptor (AR) sites. The activity level of the protein produced by an active ER or AR site is monitored while an environmental extract is introduced into the environment. Increases or decreases in protein activity suggest the presence of an EDC (Snyder, *et al*., 2003).

Receptor binding assays are useful for determining EDCs. In this method, a labeled sample protein with a known concentration has an unknown sample added to it. The known and unknown samples compete for the activation sites on the protein and the final ratio of active/inactive proteins are compared against known samples (Snyder, *et al*., 2003).

In vivo bioassays are occasionally used if more than one metabolic pathway is needed for the EDC signs to be evident. Select varieties of fish, which are sensitive to EDCs in water, are used and monitored for any changes in behavior or physiology. Several visible factors that may be affected are size and reproduction. Simple population monitoring will provide an indicator of possible pollutants in a body of water (Snyder, *et al*., 2003).

Direct measurements

Extractions are used to increase normally low concentration levels of EDCs in water samples. There are several techniques used to achieve the desired concentrations, they include: liquid-liquid, steam distillation, Soxhlet, and solid-phase extraction (SPE). These methods can be used to extract EDCs from water and solids (Snyder, et al., 2003). In a USEPA funded study, Fine, *et al.*, 2003 developed a SPE method to identify and quantify estrogens in ground water and swine lagoon samples. "Centrifuged and filtered samples were extracted using solid-phase extraction (SPE), and extracts were derived using pentafluorobenzyl bromide (PFBBR) and –trimethylsilylimidazole (TMSI). Analysis was done using negative ion chemical ionization (NICI) gas chromatography–mass spectrometry–mass spectrometry (GC–MS–MS). Deuterated analogs of each of the estrogens were used as isotope dilution standards (IDS) and were added to the samples before extraction. In swine lagoon samples from three different types of swine

operations, estrone was found at levels up to 25,000 ng/l, followed by estriol and estradiol up to levels at 10,000 and 3000 ng/l, respectively. It was found that pretreatment of swine lagoon samples with formaldehyde was necessary to prevent conversion of estradiol to estrone" (Fine, *et al.*, 2003).

Gas and liquid chromatography are used for chemical analysis in much the same manner. Gas chromatography heats a sample to evaporation, the heated chemicals move through a stationary phase where they separate according to boiling point, temperature profile, and affinity for the stationary phase. Liquid chromatography is similar except that a liquid sample is moved through a liquid phase and the analytes separate according to their movement through the liquid phase over recorded periods of time. The resulting phase is measured with ultraviolet or visible light (Snyder, *et al*., 2003).

Immunosorbent methods utilize highly specialized, tagged antibodies to mark select analytes. The radioactive tags or resulting enzyme reactions can be measured in minute amounts making this method inexpensive and highly sensitive. The high sensitivity has been extremely helpful to determine EDCs at lower concentrations (Snyder, *et al*., 2003).

Other EDC Detection Methods

Six xenobiotic hormones, a subgroup of EDCs, have been approved by the FDA for veterinary use on cattle and sheep. Concentrations of some of these hormones along with ones for human use, 17β-estradiol and ethynylestradiol, have been recorded in raw sewage, effluents of wastewater treatment plants, and in surface water. This may be due to the different sampling locations and population densities, methodology, and conditions of sampling, such as time of the year, weather, and analytical techniques. The different techniques utilized in the environmental detection of 17β-estradiol and ethynylestradiol have different precision and sensitivities. Radioimmunoassay (RIA) and Yeast Screen (YES) have a high sensitivity to measure 17β-estradiol and ethynylestradiol and both RIA and YES are useful tools to measure 17β-estradiol and ethynylestradiol in environmental waters (Kummerer, 2001, pp. 49-65).

Discussion of EDC Analytical Methods

Since EDCs represent a broad spectrum of compounds, the development of analytical techniques is challenging. Screening for contaminants based on biological activity is useful for determining the presence or absence of a particular class of compounds while the majority of instrumental techniques are used to identify and quantify specific target compounds. No single method can detect all contaminants present in an environmental sample, nor can all biological mechanisms of action be accounted for in one simple test. An integrated approach of bioassays and instrumental analyses allows for screening of many classes of EDCs. The advancement of analytical and bioanalytical technologies will improve future efforts to detect EDCs at lower concentrations (Snyder, *et al*., 2003).

EPA is currently conducting EDC risk management research. Two studies of particular interest are: *Evaluation of Drinking Water Treatment Technologies for Removal of Endocrine Disrupting Compounds* and *Analysis of Swine Lagoons & Ground Water for Environmental Estrogens*. One part of the former study involves developing analytical methods to identify and

quantify analytes. Six steroid hormones will be evaluated. In the future, a group of alkylphenolic compounds will be added. Thus far, the study has been able to develop an analytical method for the six steroid hormones. The method includes solid phase extraction followed by liquid chromatography/mass spectroscopy (LC/MS) using electrospray ionization. All six of the steroids can be separated on a C18 LC column using a single step gradient. Selective ion monitoring is being used to achieve detection limits in the low ng/L range in organic-free water. The analytical procedure for the alkylphenols is not yet complete (http://www.epa.gov/ORD/NRMRL/EDC/projects/edc_dw.htm).

The EPA project, *Analysis of Swine Lagoons & Ground Water for Environmental Estrogens*, is developing a method for analysis of low levels of natural (estradiol, estrone, estriol) and synthetic (ethynylestradiol) estrogens in ground water and swine waste lagoon effluent. The method uses negative ion chemical ionization gas chromatography/mass spectrometry/mass spectrometry. This method will evaluate the potential for ground water contamination by swine concentrated animal feeding operations (CAFOs) through land application of swine effluent wastewater or leakage from storage lagoons. Several lagoons and monitoring wells from each of two facilities (a nursery and a farrowing sow operation) have been sampled and analyzed for all four estrogens. Initial research has found that swine lagoons contain significant concentrations of natural environmental estrogens, but additional work is needed to better define analytical limits and develop storage and preservation techniques for improved sample quality assurance before an assessment of the potential for ground water contamination can be made (http://www.epa.gov/ORD/NRMRL/EDC/projects/edc_cafo.htm).

4.1.3 Ongoing Research - Analytical Methods

Research continues to help develop analytical methods for pathogens, antibiotics and EDCs. Ongoing research for analytical methods is presented in Section 4.4.

4.1.4 Conclusions

Analytical methods that test for veterinary pharmaceuticals and microorganisms found in the environment and linked to adverse human health effects were reviewed. Research studies included in this literature search focus on providing an overview of important studies for pathogens, antibiotics, and EDCs. Since there are a wide range of organisms and chemicals included in pathogens, antibiotics, and EDCs, continued research is recommended to further understand their effects on human health.

Additional research is needed to standardize analytical methods for pathogens, antibiotics, and EDCs. Standardization will allow for quality assurance and quality control procedures to be useful. In addition, a database of current research and resources provides basic information for all researchers. Research for pathogens, antibiotics, and EDCs is constantly evolving and changing. In instances that involve a wide variety of chemicals or microorganisms, an integrated approach to analytical methods should be used for environmental detection. As analytical technologies advance, the ability to locate, detect, and understand pathogens, antibiotics, and EDCs will allow for better regulation of these substances in our environment.

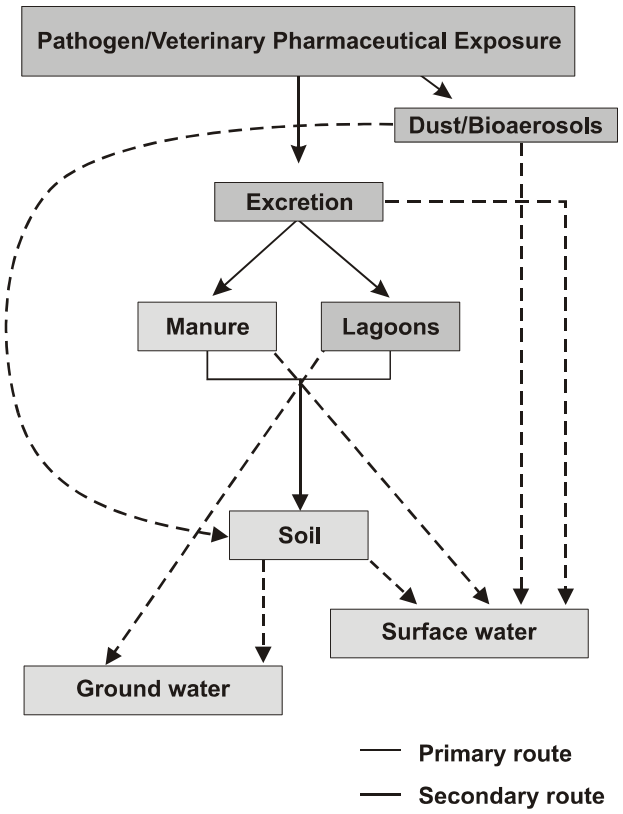
4.2 Fate, Transport, and Environmental Impacts

Air, soil, and water are directly and indirectly impacted by pathogen and veterinary pharmaceuticals from AFOs . Figure 4-1 presents the primary and secondary routes of environmental exposure. Derksen, *et al.* (2004) postulated that potential risks to aquatic species include:

- Ecotoxicological effects – shown by available biological tests (i.e., acute and chronic toxicity, genotoxicity and carcinogenicity
- Pharmacological effects – resulting from effect on non-target organism, such as interference of the hormone and immune system
- Resistance development of microorganisms.

These risks can be extrapolated to human and terrestrial species. Currently there is little information on the fate, transport, and environmental impacts of pathogens and veterinary pharmaceuticals. Available information on the existing models, research presented, and ongoing research is presented for pathogens and veterinary pharmaceuticals in AFOs.

Figure 4-1. Environmental Exposure



4.2.1 Pathogens

Pathogens are shed and present in animal feces. Table 4-2 presents incidences of five key zoonotic pathogens in cattle fecal samples. Manure management practices affect the survival times of pathogens. Temperature is probably the most important factor in determining pathogen survival times in manure. Other factors affecting survival times include ammonia, high pH, desiccation, and competition.

Table 4-2 Incidence of Key Zoonotic Pathogens in Cattle Feces (Tyrrel and Quinton, 2003)

Pathogen	Incidence in Fecal Sample
Campylobacter spp.	89 %
Listeria monocytogenes	16 %
E. coli O157	1-15 %
Salmonella spp.	23 %
Cryptosporidium spp.	48 %

4.2.1.1 Available Models for Fate, Transport, and Environmental Impacts

Literature review has shown that there are very few models to determine the fate, transport, and environmental impacts of pathogens. The following models presented include a probabilistic model to quantify nonpoint sources of pathogen production, an overview of empirical overland flow models, a probabilistic model for predicting virus attenuation, microbial flow sub-model for the Soil & Water Assessment Tool, and Hydrologic Simulation Program Fortran Model.

Probabilistic Model for Estimating the Production of Cryptosporidium spp. and Campylobacter spp.

Dorner, et al. developed a method to quantify nonpoint source of pathogenic production in a watershed illustrated with two examples – Cryptosporidium spp. and Campylobacter spp. Livestock was reported as the probable source of infection in 21% of 157 endemic cryptosporidium cases in Ontario during 1996 – 1997. Cryptosporidium and Campylobacter have been found in the following livestock: cattle, pigs, sheep, and poultry. (Dorner, *et al.*, 2004)

The prevalence of pathogenic microorganisms in animals was modeled as a mixture of β-distributions with parameters drawn from published studies. Similarly, Γ-distributions were generated to describe animal pathogen shedding intensity (Dorner, *et al.*, 2004) Study results indicate that although cattle produce the most manure, other domesticated farm animals contribute large numbers of the pathogenic organisms studied. The daily pathogen production rates are highly sensitive to the Γ-distributions.

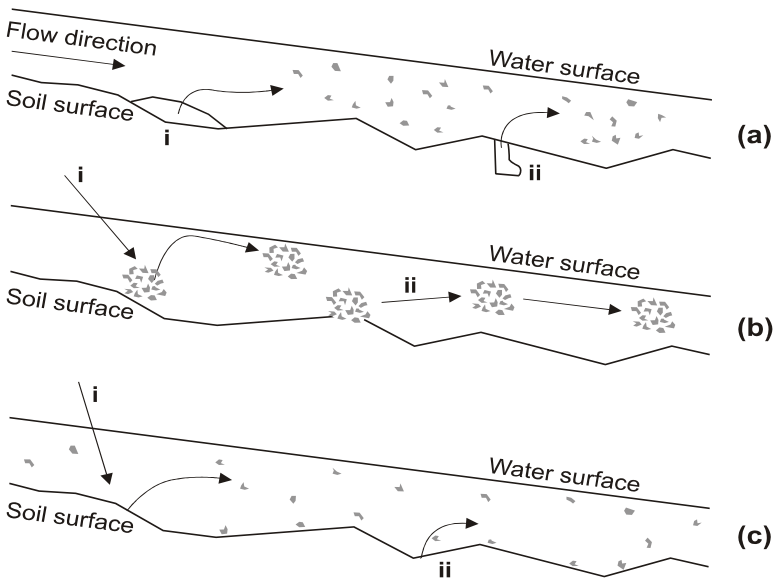
A strength of the probabilistic model is that it is a step toward better understanding pathogen sources in the environment. Unfortunately, the model results can not be compared to environmental samples as it is not possible to directly measure daily production of pathogenic microorganisms. Another weakness is that temporal variations were not considered.

Empirical Overland Flow Transport Models

Tyrrel and Quinton (2003) reviewed the current state of knowledge of pathogen transport in overland flow. Existing pathogen transport models do not describe the interactions that may occur between pathogens and soil and waste particles. Conceptual models addressing the possible states of pathogen transport have been proposed. However, the understanding of factors controlling the partitioning of microorganisms is in its infancy. Figure 4-2 presents proposed pathogen transport scenarios.

Empirical transport models are based upon the assumption that microorganisms behave like soil particles – once the population in the soil is predicted, the concentration of pathogens in the soil can be multiplied by soil loss to determine the number of microorganisms. Table 4-3 presents Tyrrel and Quinton assessment of research by Walker, *et al.*, Tian, *et al.*, and Fraser, *et al.*

Figure 4-2. Proposed Pathogen Transport Scenarios (Tyrrel and Quinton, 2003)



(a) shows the incorporation of free microorganisms in soil pores (I) and water films (ii) into overland flow; (b) shows the entrainment of waste or soil particles into overland flow as a result of raindrop (I) or flow (ii) detachment; c) shows the detachment of microorganisms from soil surfaces as a result of raindrop (I) or flow (ii) detachment

Table 4-3 Model Assessment (Tyrrel and Quinton, 2003)

Model	Description	Comments
Walker, *et al.*[1] COLI	• Used the Modified Universal Soil Loss Equation – an empirically based soil erosion model • Calculated sediment yield for a single storm based on soil & topography • Calculated number of bacteria (fecal coliform) by assuming that a constant number of cells is transported with each unit mass of manure	• No comparison was made with observed data
Tian, *et al.*[2]	• Described transport of E. Coli as a function of surface runoff volume and an empirical delivery ratio based upon landscape factors • Tian, *et al.* model compared against data	• Found that predicted and measured E. Coli were typically between 200 and 1300 CFU $100ml^{-1}$ • On one occasion sampled data was 3 times modeled output
Fraser, *et al.* [3]	• Used largely conceptual model to calculate loading rate of fecal coliforms to streams	• Model was useful in ranking relative risk of fecal contamination from livestock in 12 watersheds in NY • Correlation of predicted and observed fecal coliform concentrations in river water proved non-significant

Virulo

EPA has developed a probabilistic model for predicting attenuation of viruses during percolation in soils. "Monte Carlo methods are used to generate ensemble simulations of virus attenuation due to physical, biological, and chemical factors. The model generates a probability of failure to

[1]Tyrrel and Quinton, 2003 made assesments on information presented in Walker, S.E., Mostaghimi, S., Dillaha, T.A. and Woeste, F.E. (1990) Modeling animal waste management practice impacts on bacteria levels in runoff from agricultural lands. *Transactions of the American Society of Agricultural Engineers* 33, 807-817.

[2]Tyrrel and Quinton, 2003 made assessments on information presented in Tian, Y.T., Gong, P., Radke, J.D. and Scarborough, J. 2002. Spatial and temporal modeling of microbial contaminants on grazing farmlands. Journal of Environmental Quality 31, 860-869.

[3]Tyrrel and Quinton, 2003 made assessments on information presented in Fraser, R.H., Barten, P.K. and Pinney, D.A.K. (1998) Predicting stream pathogen loading from livestock using a geographical information system-based delivery model. Journal of Environmental Quality 27, 935-945.

achieve a chosen degree of attenuation. The model is extensible in the sense that probability density functions of parameters can be modified as future studies refine the uncertainty, and the lightweight object-oriented design of the computer model (*Virulo***)** will facilitate reuse with modified classes, and implementation in a GIS" (EPA, 2002f).

SWAT Microbial Sub-Flow Model

The SWAT (Soil & Water Assessment Tool) is a model that predicts the effect of management decisions on water, sediment, nutrient and pesticide yields with reasonable accuracy on large, ungaged river basins. However, the model does not address pathogen loadings. USDA has funded research into the development of a microbial sub-model for predicting pathogen loadings in surface and groundwater and watershed and basins. The model formulations have been structured to be comprehensive, flexible, and at a minimum contain: (1) functional relationships for both the die-off and regrowth rates and cover a range of representative values for pathogens and (2) optional processes that can be adapted to simulate the release and transport of pathogens from various sources with differing biological and physical characteristics (Sadeghi and Arnold, 2004). The model has been tested at one location and preliminary results appear to portray the general patterns of fate and transport of the pathogens. The model is undergoing further testing, development and refinement.

Hydrologic Simulation Program Fortran Model

The Hydrologic Simulation Program Fortran (HSPF) model has been used to generate a continuous flow hydrology and water quality simulation for both point and non-point source fecal coliform bacterial loading of the Polecat Creek watershed in Virginia (Im, *et al*., 2004). A variety of data, including land use characteristics, reach networks, the accumulation and deposition amounts from all known sources of fecal coliform bacteria, hydrologic verification, and meteorological data were needed for calibration. A three day test was run and compared to the model's predicted numbers. The median concentration values were considered acceptable and adjustments were made for the wash-off and in-stream degradation rates of the bacteria. More sample data was taken and the conclusions were compared. It was determined the HSPF represents the hydrology and water quality of a watershed accurately and could be used to aid in determining land use and to manage and track the resulting impact on levels of fecal coliform both in-stream and in a watershed.

4.2.1.2 Innovative Methods and Research

Following are relevant research summaries specific to ***E. coli*** and ***C. parvum***.

E. coli

Research presented are related to the genetics of antibiotic resistance and manure management practices and the effect on transport of the pathogen.

- Animal wastes containing antibiotic-resistant organisms enter the environment through the various pathways discussed. The molecular mechanisms that could potentially cause lateral or horizontal gene transfer of antibiotic resistant genes among bacteria needs to be

thoroughly understood. Integrons, which are considered to be one of the primary mechanisms for gene transfer, were researched in production and processing and in natural ecosystems. Results suggest that irrigation water and sediments contaminated with fecal waste can be sources of class 1 and class 2 integron-bearing *Escherichia coli* and the environmental factors can cause a lack of phenotypic expression in these strains. An understanding of the underlying mechanistic processes of the dissemination of antimicrobial resistance during preharvesting and processing of poultry, beef, and porcine products is critical. (Roe, 2003)

- E. coli has been shown to survive, replicate, and move downward for up to two months in soil via manure spreading or runoff from a point source. Composting is often promoted as a means of sanitizing manure to ensure that pathogenic bacteria are not spread to a wider environment. The fate of coliform bacteria during windrow composting of cattle manure from feedlot pens was examined. Numbers of total coliforms and *E. coli* declined as the composting period progressed. The duration of composting and accompanying windrow temperatures required to achieve reductions under typical field conditions were quantified (Larney, *et al.*, 2003).

C. parvum

Research is being performed at a number of institutions on the transport mechanisms of C. *parvum* in soil and groundwater.

- Bench-scale experiments to understand the transport properties of *C. parvum* have been implemented. This should provide preliminary data for a more extensive research program. Results indicate that the transport behavior is similar to that of other colloids. Experiments were conducted with different sands and at different water filtration velocity. Observations indicate that in the coarse sand the oocysts traveled approximately 20% faster than the average water molecule (called velocity enhancement in colloid science). In contrast to the colloid filtration model, low but measurable concentrations of oocysts were observed long after flushing the column with uncontaminated water. In the fine sand, oocyst concentrations persisted near the initial peak after 100 pore volumes of column flushings. This indicated a slow release of oocysts trapped in the fine sand (Harter, 2003)

- A quantitative process-based understanding of the fate and transport of *C. parvum* that is relevant to oocyst transport at the laboratory and small field scale that can be used to estimate transport in porous media was developed. They concluded that the clean bed filtration model without detachment provides an excellent tool to predict maximum oocyst concentrations following a contamination event. However, contaminated porous media become a significant source of oocycsts potentially leading to long-term low-level elution of C. parvum oocysts. (Harter *et al.*, 2000)

- Vegetated buffer strips placed between animal agricultural operations and surface water supplies are being advocated to substantially reduce the survivability and off-site transport of infective *C. parvum* oocytes from animal manure. Vegetated buffer strips were evaluated for their ability to remove waterborne *C. parvum* from surface and shallow subsurface flow during simulated rainfall. Sandy loam or soil with higher bulk density were less effective at

removing *C. parvum* compared to silty clay or loam or at lower bulk densities. Filtration efficiency was conditional on combinations of slope and soil conditions. Atwill, *et al.* recommended the size and design of vegetated buffer strips, assuming active maintenance for minimizing overland and subsurface preferential flow paths, for events involving low to moderate precipitations. (Atwill, *et al.*, 2002).

- Transport behaviors of *C. parvum* in aquifers were studied using experimental investigations and numerical modeling. A numerical model simulating the transport phenomena of oocysts under the experimental conditions of assays (continuously recirculating column and open column) was developed to establish the whole experimental curve of results using a small number of experimental points. The continuously recirculating assays provided satisfactory results, the numerical simulation was correct and its use in result interpretation was relevant. They believe that it is possible to determine transport parameters by matching experimental points with numerical results. (Marly, *et al.*, 2001)

- The dispersion and initial transport of Cryptosporidium oocysts from fecal pats as a function of watershed characteristics such as slope, presence of vegetation, and rain event intensity and duration was quantified. Intact soil blocks were utilized to provide a more realistic simulation of overland oocyst transport and by infiltration under natural conditions. The quantitative data derived fro Cryptosporidium oocyst dispersion and transport from fecal pats should facilitate the construction of models to predict source water quality and to better manage the factors that govern pathogen transport within watersheds (Davies, *et al.*, 2004)

4.2.1.3 Ongoing Research – Pathogens

Current research is presented in Section 4.4. The majority of the ongoing research is focused on modeling the fate and transport of pathogens through the soil and water. Manure management practices are also being assessed.

4.2.1.4 Future Research Needs

Following is a list of suggest research culled from the review of the research presented.

Campylobacter

- Identify sources and temporal factors associated with pathogen shedding and survival

E. coli

- Role of quorum sensing on exposure to sub-therapeutic antibiotic concentrations acquisition of antibiotic resistance
- Impacts of specific poultry processing steps on retarding the transfer or resistance determinants
- Pathogen transport mechanisms – dynamics and factors affecting it

C. parvum

- Further research of transport properties
- Experimental and theoretical research to measure and explain the long-term elution behavior of *C. parvum*.
- Studies on fate and transport of zoonotic protozoal pathogens in overland and subsurface flow through vegetative buffers
- Determine design criteria for on-farm vegetated buffer strips for waterborne microbial contaminants
- Further develop the numerical model
- Vegetative buffer strips – further research on 1- to 20-m travel distance with successive rain events and measurements of soil saturation to investigate the potential travel times for mobile or remobilized oocysts from land to waterways

Overall, research is necessary to increase the knowledge base in the sources of pathogens, effect of natural processes on pathogens, development of pathogen environmental fate and transport models, transport properties, and manure management practices to reduce the transport of pathogens.

4.2.2 Veterinary Pharmaceuticals

Environmental exposure to veterinary pharmaceuticals (e.g. Antibiotics, EDCs) occur through similar emission and distribution routes even though there are varying uses. Veterinary pharmaceuticals are ingested, injected, and topically applied. The major uses of veterinary pharmaceuticals are as growth promoters, antibacterial agents, and antiparasitic agents. Antibiotics and EDCs are the pharmaceuticals of concern addressed in this discussion. Currently, research has only been performed on human pharmaceuticals that are used as veterinary drugs.

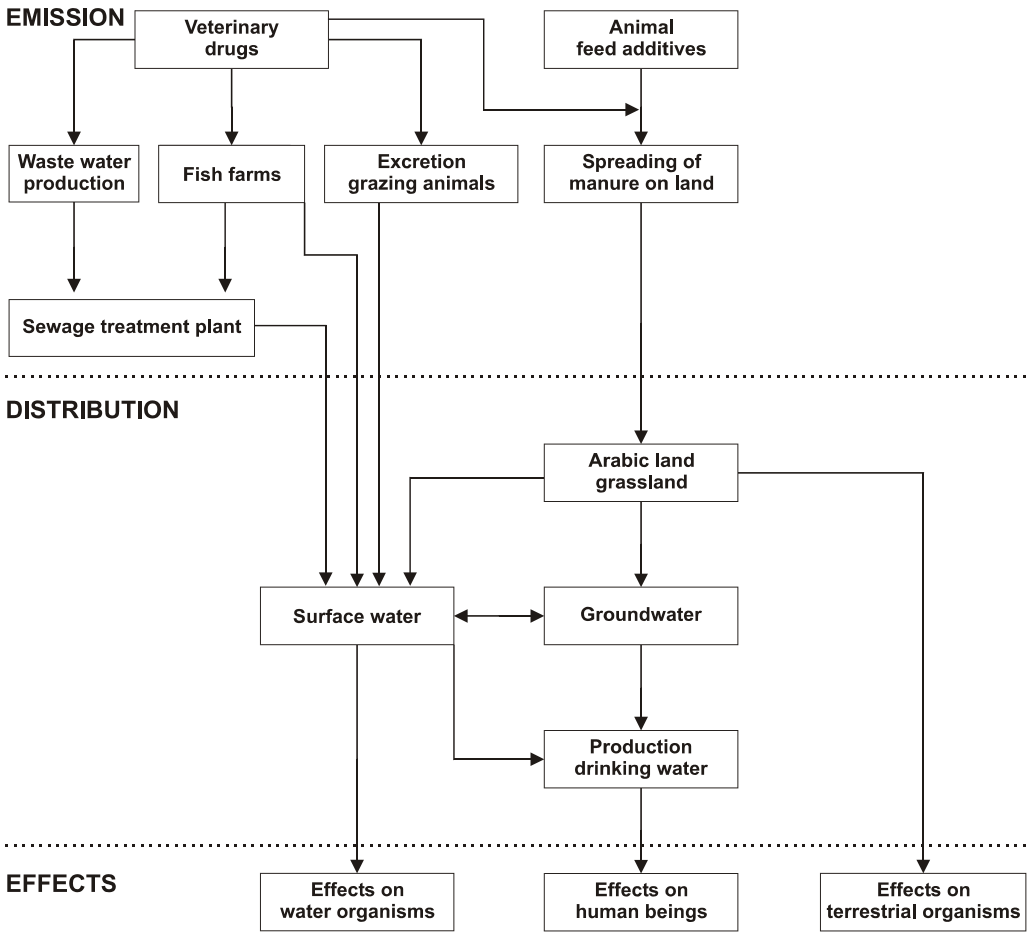
Veterinary pharmaceuticals can enter the environment through a number of routes including:

- removal and subsequent disposal of waste materials
- via excretion of feces and urine by grazing animals
- through spillage during external applications
- direct exposure/discharge to the environment
- contamination of soil column
- leaching to shallow groundwater from manured fields
- surface water bodies through surface run off
- through topical applications wash off to surface water and soil
- air borne contamination through topical applications

- biota uptake

Figure 4-3 depicts the fate (emission, distribution, and effects) of veterinary pharmaceuticals and feed additives.

Figure 4-3. Fate of Veterinary Drugs and Animal Feed Additives (Derksen, *et al.*, 2004)



4.2.2.1 Available Models for Fate, Transport, and Environmental Impacts

Available models for fate, transport, and environmental impacts of veterinary pharmaceuticals are limited. Currently there is a focus on identifying the available models through literature reviews. They have shown that there are few models available to evaluate the environmental distribution and fate of pharmaceuticals in the environment. There is a lack of data and test methods appropriate and specific for these substances.

In Kummerer (2001), Di Guardo, *et al.,* proposed a methodology to evaluate the distribution and fate of pharmaceuticals. They propose four stages:

- Data Evaluation – Collection and critical assessment of structural formulas and physical/chemical data (molecular weight, vapor pressure, solubility in water, K_{ow}, and pK_a. Discharge and emission patterns are necessary.
- Use of Generic Models – Necessary to understand the main environmental pathways in a generic predefined environment (i.e. water only, air only, soil only). Will give information on the mobility and overall persistence in the environment.
- Use of Regional Models – Collection of regional environmental data and simulations in regional scenarios.
- Use of Site Specific Models – Used to predict environmental concentrations in a specific medium based upon regional modeling.

The goal of the proposed strategy is to use modeling approaches to understand the fate and distribution of pharmaceuticals in the environment. Modifications would be required to adapt the models to the specific pharmaceutical and exposure mechanism.

Boxall (2000) reviewed a range of models for predicting the fate of compounds entering soils, the aquatic environment, and air (see Table 4-4). The models presented are primarily for pesticides and used in the pesticide registration process. These models have been recognized for use by FOCUS and/or recommended by the FIFRA Exposure Modeling Work Group (Boxall, 2000). Further study would need to occur to determine the applicability of the models listed in Table 4-4.

Table 4-4 Selected Models that Could Be Used for Predicting Exposure Concentrations for Veterinary Medicines (Boxall, 2000)

Soil	Runoff/Drain Flow	Leaching	Surface Water Fate
FEDESA Model	PRZM-2	MACRO	EXAMS
PERSIST	PELMO-2	PRZM-2	SLOOT BOX
	GLEAMS	PELMO-2	ABIWAS
	EPIC	GLEAMS	WASP
	GENEEEC	PETLA	GENEEC
	RICEWQ	LEACHM	RIVWQ
	MACRO	PATRIOT	
	PESTLA	CHEMRANK	
		CMLS	
		SCIGROW	
		PESTAQ	

Estimates of the likely uptake of a substance, such as veterinary pharmaceuticals, by biota from water, sediment, air and soil can be obtained based on knowledge of the substances physical/chemical properties. These relationships are based upon a compounds octanol-water

partition coefficient. Biotic fate models, not pharmaceutical specific, have been developed. They generally include a trophic structure and a description of the chemical fate of a contaminant amongst the biotic components (Boxall, 2000).

Risk Assessment

The European Union has issued directives that require Environmental Risk Assessment for Veterinary Medicinal Products other than GMO-containing and Immunological Products be performed – European Directive 2001/82/EC. A guidance document details the assessment of the potential of exposure of the environment to a veterinary medicinal product, its ingredients and relevant metabolites and the assessment of potential harmful effects which the use of the product may cause to the environment (European Communities Commission, 1997). The required risk assessment has two phases. Phase I assesses the potential environmental exposure to the product, its ingredients, or metabolites. Phase II is a two tiered approach to determining the extent of environmental exposure to the product, and the available information about the physical/chemical, pharmacological, and/or toxicological properties. If during Tier A, further study is warranted, Tier B studies relating to the effects on fauna/flora need to occur.

To support the registration of veterinary medicinals, the Netherlands has prepared a report and methods to guide applicants and the national registration authority through the evaluation scheme. "It contains transparent exposure models that predict exposure concentrations, as well as, uniform guidance to assess the potential effects of the product to exposed organisms in dung, soil and water. If reliable actual exposure data are available, these may replace the predicted values" (RIVM, 2003). Montforts (2003a) gives guidance on recommended methods for the risk assessment of veterinary medicinal products applied in slurry. Fate testing strategies, effects testing strategies and assessments, and scenario definitions using FOCUS are addressed. In Montforts (2003b), the validity of exposure and distribution models for soil, ground water and surface water are assessed. The functional validations with (oxy)tetracycline and sulphonamides indicate that it is impossible to analyze the contribution of every single model parameter to the variability in the model predictions using random field samples (Montforts, 2003B). "Animal husbandry, slurry handling, and environmental conditions throughout Europe are considered in order to define realistic worst case scenarios, in conjunction with environmental distribution models. Scenarios and models for distribution of surface and groundwater provided by FOCUS are considered suitable for veterinary drugs" (Montforts, 2003c).

4.2.2.2 Ongoing Research – Antibiotics and EDCs

Current pharmaceutical fate studies have been survey-oriented, only documenting occurrence in a variety of environmental systems. Temporal and spatial distribution data and environmental degradation rates of pharmaceuticals have not been collected in the US (MacKay, 2004). Literature surveys show that the primary focus of current research is on Human Pharmaceuticals in the environment.

Two studies on EDCs are:

- A study utilizing fathead minnows was conducted to study the differences in the reproductive biology between a groups of minnows from a stream directly below the effluent outfall from a feedlot, from a stream that receives runoff from an agricultural field with disbursed cattle, and from non-contaminated areas upstream from the two previous sample areas. The size, sex hormone levels and gonads of the sampled fish were tested for the effects of Trenbolone-β, an active synthetic anabolic steroid. The female fish near the contaminated areas were found to have higher levels of androgens in their systems and smaller distances between internal organs than those from upstream. Similarly, male minnows had smaller testicles and closer internal organs than those from non-contaminated waters. No pathology was apparent in the ovaries or testicles of the fish collected in the contaminated water (Orlando, *et al.*, 2004).

- EPA has studied the potential of CAFOs to contribute estrogens to the environment. Environmental estrogens refer to a wide range of anthropogenic or naturally occurring compounds that elicit estrogenic responses by mimicking endogenous estradiol. A method was developed for analysis of low levels of natural and synthetic estrogens in ground water and swine waste lagoon effluent. The results show that swine lagoons contain significant concentrations of natural environmental estrogens, but additional work is needed to better define analytical limits and develop storage and preservation techniques for improved sample quality assurance before an assessment of the potential for ground water contamination can be made (http://www.epa.gov/ORD/NRMRL/EDC/projects/edc_cafo.htm).

Ten ongoing research studies were identified that were specifically related to AFOs and veterinary pharmaceuticals (see Section 4-4 for descriptions). Of the ten studies, three studies were for class of veterinary pharmaceuticals, five were for antibiotics, and one was for EDCs. Table 4-5 lists the study, compound studied, and applicable media.

Table 4-5 Veterinary Pharmaceuticals Ongoing Research

Study	Compound	Media
Hapeman, USDA	Veterinary Pharmaceuticals	Air
Kee Ong, USGS	Antibiotics	Water
Pedersen, USGS	Antibiotics	Soil
EAWAG	Antibiotics	Water
EAWAG	Antibiotics	Soil
Lee, CSREES IND	Veterinary Pharmaceuticals	Soil, Water, Air
MacKay, CSREES CONR	Antibiotics	Soil
Lee, CSREES IND	Antibiotics, Hormones	Soil, Water
Lee, CSREES IND	Veterinary Pharmaceuticals	Soil
Kenimer, TAMU	EDCs	Soil, Water

4.2.3 Recommendations for Further Research

Research specific to AFOs and Veterinary Pharmaceuticals is lacking. Research is needed in the following areas (Boxall, 2001), (Kummerer, 2001), (Derksen, et al., 2004).

- Usage Data – Many groups are unavailable. Usage data is necessary for properly designed and science-based effective risk assessments.
- Emission Routes – The significance of routes of exposure are unknown. Once the routes are assessed they can be incorporated into risk assessment models as appropriate.
- Ecotoxicity Studies – Limited number of terrestrial species have been tested. Some aquatic species have been tested, but further testing is necessary. This will identify high risk groups and specie sensitivities, distributions, and impacts on aquatic and terrestrial health.
- Endocrine Disrupting Potential of Hormones and other Substances – There is limited information available. The information when combined with releases will enable the assessment of potential for disruption of species endocrine systems in the environment.

4.3 Strength of Evidence Linking Outbreaks to AFO Contaminants - Importance of Locating the Source of Contaminants

As discussed in Section 4.1, AFOs introduce many pollutants into the environment. Untreated or improperly treated animal manure may contain pathogens that can reach food crops or water supplies (USEPA, 2004). Although other sources can contribute pathogens to the environment, AFOs contribute a major portion of the pathogenic contamination in most watersheds (Pell, 1997). These pathogens can be transmitted to humans via food or water supplies and present a significant risk to human health. Table 4.1 provides a list of pathogens common to farms and the corresponding human disease.

Determining the source of an outbreak is important because it allows health officials to limit the number of people impacted by an outbreak, and it allows enforcement officials to hold the offender accountable for the contamination. Widely dispersed foodborne outbreaks are a major concern because the world's food supply continues to be mass produced and widely distributed (Gill, *et al.*, 2003). In addition, fecal contamination of water bodies causes impairment of drinking, fishing, or recreation. Waters are qualified as impaired when indicator microorganisms reach levels that indicate that pathogenic bacteria are also present in unsafe levels (Scott, *et al.*, 2002). Once a water body is qualified as impaired, a Total Maximum Daily Load (TMDL) may be initiated. A TMDL is the calculation of the maximum amount of nonpoint contamination that a water body can receive and still meet water quality standards (Hartel, 2002). Creating a TMDL for an impaired water body is time consuming and expensive. Preventing a water body from becoming impaired will reduce costs.

Since food and water supplies can be contaminated by several sources, differentiating between these sources will help locate and reduce fecal pollution. Locating the source of foodborne and waterborne pathogens within a watershed is difficult because they can come from many different

sources including human wastewater treatment plants, animal feeding operations, or wildlife. This literature search examines evidence for tracing the path of an outbreak from the pathogen that caused the human illness back to the source of that pathogen, whether it be from humans, production animals, or wildlife.

4.3.1 Methods for Tracing Diseases to AFO Contaminants

Since fecal pollution can come from several sources, tracing the pathogens back to their exact source is challenging. Based on the need to distinguish between sources of fecal pollution, new fecal source tracking methodologies have emerged.

There are many methodologies for fecal source tracking that can be categorized into several groups. These methodologies can be used individually or in combination based on the desired outcome of research. The categories for fecal source tracking are: microbiological methods, genotypic methods, phenotypic methods, and chemical methods (Scott, 2002; Simpson, *et al.*, 2002). Strengths and weaknesses for each methodology are discussed in Table 4.6. A short description of each methodology follows:

- *Microbiological Methods* - methods that use the microbiology of a pathogen. Examples: fecal bacteria ratios, F-specific (F+ or FRNA) coliphage, and human enteric viruses.
- *Genotypic Methods* - methods that distinguish among bacterial and/or viral samples based directly on their genetic makeup. Examples: Pulse Field Gel Electrophoresis (PFGE), host-specific molecular markers, ribotyping, and repetitive element polymerase chain reaction (PCR).
- *Phenotypic Methods* - methods that distinguish among samples based on secondary characteristics, such as antibiotic resistance. Examples: antibiotic resistance analysis (ARA) or multiple antibiotic resistance (MAR), immunological methods, sterols or fatty acid analysis, and nutritional patterns.
- *Chemical Methods* - methods that are designed to detect chemical compounds associated with humans. These chemicals are often found in wastewater such as septic tank effluent. These methods do not detect fecal bacteria. Examples: coprostanol, optical brighteners, and caffeine detection.

One methodology, microbial source tracking (MST) (also referred to as bacteria source tracking or BST), uses indicator organisms to detect subtle differences present within different groups of microorganisms. These differences identify the host or environment from which the indicator organism is derived (Scott, *et al.*, 2002). MST is an evolving science that has not received a large amount of funding until recently, corresponding with the implementation of the TMDL Rule by EPA in 1992 (http://soils1.cses.vt.edu/ch/biol_4684/bst/BSTmeth.html). MST is one tool for determining sources of fecal pollution and should be used for projects that involve TMDL development for fecal coliforms, or in the design and implementation of best management practices to reduce fecal loading in water (http://soils1.cses.vt.edu/ch/biol_4684/bst/BSTmeth.html). As more studies are conducted and MST becomes refined, more information about its reliability will emerge.

Fecal source tracking research often begins with epidemiological data. Researchers conduct interviews with infected patients to discover the common source of an outbreak, whether it is foodborne,

waterborne, human-to-human contact, recreational, or direct contact with livestock (Peng, *et al.*, 1997). This research allows scientists to narrow their search for the source of an outbreak. This type of study does not scientifically prove a contamination source, however, it provides critical information for beginning laboratory research. Another method used by researchers is the Livestock Density Indicator (LDI). Valcour (2002) used LDI to examine the correlation between livestock density and cases of human Shiga toxin-producing *Escherichia coli* (STEC) infection. The study found a higher incidence of human STEC infections in rural areas and a spatial association between the incidence of human STEC infection and cattle density. Spatial analytic techniques provide another means of identifying populations at risk for pathogen infections. These studies, however, do not locate the exact source of contamination that causes an outbreak.

4.3.2 Research Findings

Current research provides information on the ability to use fecal source tracking to link human outbreaks of disease and AFO contaminants. After reviewing the studies available, three types of studies emerged. First, several studies used fecal source tracking to link an outbreak to a water supply (Waterborne Outbreak Studies). These studies found that human diseases were linked to a common water source, and that the water source was most likely contaminated by an animal. These studies, however, did not define the specific animal source, whether it be a production animal or wildlife. In most cases, speculation is made based on epidemiological data. Second, several studies linked an outbreak to a food source (Foodborne Outbreak Studies), but did not explain how the food source was contaminated. Again, in some cases, epidemiological data is mentioned to provide a guess at the source. Finally, one study was able to directly link AFO contaminants to human incidents of disease (Study that links outbreak to AFO contaminants). These three categories of studies are discussed in more detail in the following sections.

4.3.2.1 Waterborne Outbreak Studies

Several studies suggest the connection between an outbreak from a water supply and animal contaminants but can not conclusively connect the two. In these studies, fecal source tracking is used to determine that the source of the outbreak is a water supply, but does not locate the specific animal or farm that contributed the contaminants. Epidemiological information is used to suggest a source for the contamination. Four waterborne outbreak studies are summarized below:

- An investigation of a *Cryptosporidium* waterborne outbreak in March 2000 in northwest England using genotyping confirmed that genotype 2 was present, which is usually associated with animals. Environmental site inspections suggested that contamination of water supplies with animal feces was the probable cause of the outbreak. Recent cattle excreta were present in and around the covers of several spring collection chambers and that manure was spread on a field within 5m of a wellhead. No manure samples were taken, however, to confirm this speculation (Howe, *et al.*, 2002).

- Glaberman (2002) investigated three unrelated drinking water outbreaks of *Cryptosporidium* in Northern Ireland from April 2000 to April 2001 using PCR-RFLP. Two outbreaks were caused by a human genotype and a third by a bovine genotype. The human genotype outbreaks were caused by raw sewage and wastewater leaking into drinking water distribution systems and was supported by subgenotyping a wastewater sample; it contained a subgenotype of the *C. parvum* genotype

indistinguishable from the one found in infected persons. No animal samples were taken or analyzed to confirm the bovine genotype outbreak.

- Olsen, *et al.* (2002) conducted a study of a 1998 *E. coli* outbreak in Wyoming using sorbitol-MacConkey agar and PFGE. Although the study concludes that the outbreak was caused by the municipal water supply, there is no connection to a contamination source. The only hypothesis is that deer and elk feces seeped into the water supply. A small number of samples of the deer and elk feces did not yield *E. coli*.

- A study of a waterborne outbreak of *E. coli* and *Campylobacter* in New York in August 1999 uses PCR and PFGE to determine the source of the outbreak. The source of the outbreak was the water distribution system with the probable source of contamination coming from cow manure that seeped into a water well. Six samples of cow manure, however, were unable to confirm this hypothesis (Bopp, *et al.*, 2003).

4.3.2.2 Foodborne Outbreak Studies

Pathogens can also contaminate our food supply. In these studies, the food source of the contamination is clear, but there is no link to an AFO (or other) contamination. Five studies of foodborne outbreaks are summarized below:

- Samples taken by the Maryland Department of Natural Resources found that oysters in the Chesapeake Bay for human consumption contain high levels of *Cryptosporidium* oocysts. Evaluation of DNA using the polymerase chain reaction (PCR) technique yielded both a human and bovine genotype. Therefore, portions of the Chesapeake Bay contained human or animal feces when oysters were filtering water and they were able to capture the oocysts. No connection was made to a specific farm or animal type. The Chesapeake Bay watershed has 185 million livestock animals that excrete 44 million tons of manure each year (Chesapeake Bay Foundation, 2004).

- In a study by Rabatsky-Ehr, *et al.* (2002), DNA fingerprinting by PFGE and serotyping confirmed that a human outbreak of *E. coli* came from deer meat in Connecticut. Researchers speculated that the deer acquired *E. coli* from cows grazing on dairy farms in Vermont, but no samples confirmed the speculation.

- A comprehensive study of *E. coli* and *Salmonella* outbreaks on raw seed sprouts discussed the advantages of subtyping isolates, including serotyping and molecular typing (PFGE) to determine whether clusters of infections across states are related and provide a link to a common source (seed lot). A national electronic subtyping network has been established for *E. coli* that will allow participating states to rapidly compare DNA PFGE patterns of *E. coli* strains with the patterns in a national database. No connection was made to a contamination source (Taormina, 1999).

- A study of an outbreak of *E. coli* from alfalfa seeds in June and July 1997 in Michigan and Virginia traced contamination to a single seed lot in Idaho. The *E. coli* isolates from outbreak participants had an identical PFGE pattern, the same phage type, and the same antibiogram, which strongly suggests a common source. Site inspection at the Idaho facility yielded three possible sources of contamination: cattle manure, irrigation water, or deer feces. No samples were taken to confirm observations (Breuer, *et al.*, 2001).

- A multi-state outbreak of *Salmonella* on alfalfa seeds in 1999. All *Salmonella* isolates from outbreak patients were serotyped; PFGE supported genetic relatedness of *Salmonella* isolates. The outbreak was traced back to a single seed lot. No confirmations were made about seed contamination, however, an inspection of the farm showed numerous opportunities for contamination by wild and domestic animals or river water (Gill, *et al.*, 2003).

Mass produced and widely distributed food can contribute to an increasing number of widely dispersed outbreaks. Subtyping tools; centralized disease reporting and outbreak investigation; application of appropriate microbiologic tests; a common, international language; and an increase in awareness are critical for reducing outbreaks (Gill, *et al.*, 2003; Taormina, 1999).

4.3.2.3 Study that Links an Outbreak to AFO Contaminants

One study links a human outbreak of disease to AFO contaminants. A study of an *E. coli* and *Campylobacter* outbreak in Walkerton, Canada in 2000 used both genotypic and phenotypic methods to discover that bacteria entered the municipal water supply from neighboring farms. The results pinpointed a specific farm as the culprit of the contamination. The methods used in this study were: serotyping, phage typing, biotyping, and PFGE (Clark, *et al.*, 2003).

4.3.2.4 Discussion of Studies and Methods

Since only one study has emerged that can conclusively link an outbreak to AFO contaminants, research needs to continue on the various methodologies to determine the best method or methods for tracing fecal contamination. Most likely one single method will not be able to make the connection, and instead several methods will emerge as the best choice for tracing an outbreak back to its source. By combining several methods, results will be strengthened. Non-molecular methods (phenotypic and chemical) are advantageous early in fecal source tracking to quickly discriminate human from animal sources. Also, non-molecular methods are usually inexpensive and can be performed on hundreds of isolates per week. Molecular methods (genotypic) can be used later in the process to validate results because these methods can show the unique differences in DNA of fecal bacteria. These methods can match the DNA of an outbreak organism to the DNA of an organism in the manure of a production animal.

Some studies have produced results that question the original assumptions of the methods. Studies by Peng, *et al.* (1997) and Xiao, *et al.* (2001) reference two distinct genotypes for *C. parvum* in humans rather than only one genotype as previously thought. In past studies, characterization of *C. parvum* has relied on two genotypes, one specific to humans and the other to animals. This finding is important for watersheds that removed cattle as a management strategy for reducing pathogens. Human wastewater may also be contributing to pathogen contamination and subsequent outbreaks. In addition, the association of *Salmonella* genotypes with human and animal feces may not be as absolute as previously thought (Schaper, *et al.*, 2002). Two genotypes were thought to be associated with humans (genotypes II and III) and two different genotypes with animals (genotypes I and IV); however, samples from hospital wastewater, raw sewage, abattoir wastewater, and animal/human feces found significant levels of the incorrect genotypes. This information indicates that the association of genotypes with human and animal excreta is statistically significant, but not absolute (Schaper, *et al.*, 2002). These findings will need to be examined further with additional research and kept in mind when reviewing future studies.

Other studies add insight about different methodologies for fecal source tracking. A summary of each follows:

- In an EPA study, "Detection of Pathogens in Drinking Water," a technique was developed to simultaneously detect animal cells (sheep, cow, human, or horse) and *E. coli* 0157:H7 using PCR. Results detected and differentiated the presence of human and horse or cattle, but not sheep DNA samples (http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/6038/report/0).

- EPA has developed a method of fecal source identification based on PCR amplification of marker sequences from uncultured Bacteroidetes. Fecal anaerobic bacteria can rapidly and accurately identify sewage and feces from humans, cattle and other ruminants, dogs, pigs, horses, and elk. However, few or no known cultured species of *Bacteroides* and *Prevotella* can distinguish human from animal feces, because humans, dogs, cats, and gulls share closely related sequences. The Bacteroidetes method of fecal source identification compared favorably with other methods in a comparative study. Of the three methods that performed well in the study, including ribotyping and PFGE, the Bacteroidetes method is the least expensive and most rapid (http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/279/report/0)

- The bacteria, *Enterococcus faecalis*, has been suggested as a superior bacterial indicator of fecal contamination for recreational waters. In an EPA study, a 16S rDNA real-time PCR method was developed to detect *E. faecalis* in both a water distribution system and in freshwater samples. This method has the potential to for providing rapid detection of *E. faecalis* in water compared to other phenotypic identification methods that can require up to 72 hours for results and are sometimes inaccurate (Santo Domingo, 2003).

- EPA performed a study assessing equine fecal contamination as an alternative bacterial source tracking target. Novel sequences from *Bacteroides* and *Prevotella* analysis produced an equine specific phylogenetic cluster as compared to previous data sets obtained for human and bovine samples. The results of this study suggest that anaerobic fecal bacteria may be potential identifiers for use in source-tracking applications. However, a comprehensive examination of anaerobic environmental sequences within these species should be performed before methods targeting these bacterial groups are applied to watersheds on a large-scale (Simpson, et al., 2004).

- EPA conducted a study to develop, evaluate, and apply new and improved methods to detect *Cryptosporidium*, *Salmonella* spp., *Yersinia enterocolytica*, and indicator viruses (somatic and male specific coliphages) in water and wastes. As the methods were developed and refined, some were applied to the determination of pathogen and indicator levels in waters impacted by both point and non-point sources of fecal contamination in watersheds and aquifers. Some of the methods used were: EPA Method 1622, molecular methods (DNA-RNA chemiluminescence), and bioassays (http://cfpub.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/607/report/F)

- EPA conducted a study to determine the prevalence of fecal shedding, distribution of genotypes, and associated risk factors for *C. parvum* infection in populations of feedlot cattle in the United States

using immunomagnetic separation followed by direct immunofluorescent microscopy (IMS-DFA). Of the nine DFA+ and two IMS-DFA+ samples, four isolates had sufficient numbers of oocysts to allow molecular confirmation as *C. parvum*. Using a nested polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, the genotype was bovine genotype A (http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/822/report/F)

4.3.3 Recommendations and Future Research

Further research will help answer some of the questions about the strengths of methodologies and streamlining the process for source tracking. One suggestion for future research is to create a national library of *E. coli* isolates for humans and animals to help track and identify fecal pollution. The library of isolates will help determine whether several infections across states are related (Taormina, 1999). Another suggestion for research is to compare fecal source tracking methodologies to create a standardization of methods because of the lack of uniform standards or reference materials (Bernstein, *et al.*, 2002). Additional studies comparing methodologies will help with problems related to detection limits, temporal and spatial variability of markers, and reproducibility of assays (Simpson, *et al.*, 2002).

Many fecal source tracking research projects are being conducted around the country. Since the results have not been published, they were not included in the previous sections. These studies can provide valuable insight about the direction of research. A list of ongoing research projects are found in Section 4.4.

4.3.4 Conclusions

Fecal source tracking can be used to determine the source of contamination from foodborne and waterborne outbreaks. In at least one study, fecal source tracking has been successful in linking a human outbreak to AFO contamination. The MST methodology will continue to gain popularity as TMDLs become increasingly important. The studies conducted to date show high potential for having the ability to turn a nonpoint source into a point source. This will allow for a more accurate assignment for waste load allocations and the development of a scientifically-defensible TMDL (CSREES, 2004). Fecal source tracking can also be used to quickly and effectively locate the source of a outbreak in order to destroy it before additional people become sick. Locating the source of an outbreak quickly, whether it be foodborne or waterborne, can help health officials warn others that might be exposed to take precautions to avoid becoming sick. Future research will help strengthen the viability of methodologies and streamline the process for source tracking.

Interestingly, because of the diversity of animal types and intrinsic evolutionary processes in microbial organisms, these methods will never be completely accurate. The challenge for any research is to recognize the patterns that cannot be classified and determine when an increased effort to refine methods and build libraries produces diminishing returns (Bernstein, *et al.*, 2002). This information and the research cited suggest that no one single method is capable of identifying the source of contamination for an outbreak. Several studies have suggested using a “toolbox” approach to studying the source of contamination in order to speed up the process in certain instances and verify results in other instances. As more studies are conducted, the advantages and disadvantages of each fecal source tracking method will become clear. This will allow for the best possible resources for determining the source of human outbreaks from AFO contamination.

Table 4-6 Fecal Source Tracking Methodologies, Strengths, and Weaknesses (Information taken from http://soils1.cses.vt.edu/ch/biol_4684/bst/BSTmeth.html, unless otherwise indicated)

Method	Description	Strengths	Weaknesses
Microbiological Methods - methods that use the microbiology of a pathogen			
Fecal Bacteria Ratios	Based on the ratios of different stomach and intestinal bacteria, not just fecal coliform bacteria. Develop a ratio coefficient that is useful in source identification.	--Provide rapid results. --Requires minimal expertise (Scott, 2002).	--Traditional fecal coliform-fecal streptococcus ratio is no longer reliable for source identification, instead, ratios could be used as a general indicator of human vs. non-human fecal bacterial contamination. --Based on unstable parameters and may thereby lead to erroneous conclusions (Carson, 2001).
F-Specific (F+ or FRNA) Coliphage	Coliphages are viruses that infect *E. coli* that can be differentiated using serology. There are four antigenically distinct serogroups of FRNA coliphages, and those predominating in humans (groups II and III) differ from those predominating in animals (groups I and IV). It may be possible to distinguish between human and animal wastes by serotyping FRNA coliphage isolates.	--Useful early in investigation, due to the speed of results (Clark, *et al.*, 2003).	--Pigs contain genotype II. --Not all animals have FRNA coliphage associated with their *E. coli*. Coliphage remains in environment for less than a week and survival is a function of sunlight and water temperature. DNA fingerprinting of FRNA coliphages may resolve some of the problems with serological typing.
Human enteric viruses	Cultivation of human enteric viruses present in the human gastrointestinal tract.	--Can quickly determine human contamination. --Avoids uncertainty	--Useful only for determining human fecal contamination. --Many viruses can be present in a water system, while only a

Method	Description	Strengths	Weaknesses
Genotypic Methods - Locating the unique differences in the genetics (DNA) of fecal bacteria (human and animal) by a variety of methods. Referred to as "DNA fingerprinting" and are based on the unique genetic makeup of different strains, or subspecies, of fecal bacteria			
Pulse Field Gel Electrophoresis (PFGE)	Generates DNA fingerprinting by treating genomic bacterial DNA with rare-cutting restriction endonucleases (Scott et. al, 2002)	–Shows unique differences in DNA of fecal bacteria.	--Few studies, research is developing.
Host-Specific Molecular Markers	Detection of host-specific molecular markers in raw water samples to characterize a microbial population without first culturing the organisms.	--Effective method for characterizing a microbial population without first culturing organisms. --Provides for a more rapid identification of organisms (Scott 2002).	--Little is known about indicator organisms (Bacteroides spp.). --Many organisms do not contain toxin or adhesion genes regardless of their host specificity (Scott, 2002).
Ribotyping	Bacterial genes that code for ribosomal RNA. Because such genes are highly conserved in microorganisms, ribotyping has been widely accepted for microbial identification.	--Effectively tracks human and non-human sources (Scott, 2002). --Human and animal ribotypes are significantly different and useful in identifying fecal pollution (Carson, 2001).	--Expensive, detailed and time-consuming procedures, and not yet suitable for assaying large numbers of samples in a reasonable time frame Success depends on the size of the “known-source” reference fingerprint database (Scott, 2002). --Fingerprint can change with the diet of the animal (Scott, 2002).
Repetitive element polymerase chain	Primers correspond to interspersed repetitive DNA elements present in various locations within prokaryotic genome to generate highly specific genomic	--Useful in creating a database of patterns to compare with unknown sources	--PCR inhibitors in the extracted DNA may prevent the detection of PCR (Glaberman, 2002)

Method	Description	Strengths	Weaknesses
PCR-Restriction Fragment Length Polymorphism (RFLP)	RFLP cuts a selected segment of DNA into fragments using restriction enzymes, which only cut the DNA at specific locations. The differing DNA fragment lengths are separated by electrophoresis and visualized by staining procedures. PCR is used to make copies of the target gene sequence. (USEPA and CDC, 2002).	--Faster and less costly approach to characterizing DNA. --Detects and differentiates species of Cryptosporidium (USEPA and CDC, 2002).	--A less detailed approach to characterizing DNA. --Can not conclusively prove the animal source (USEPA and CDC, 2002).
Phenotypic Methods - methods are based on an effect of an organism's genes that actively produce a biochemical substance. The type and quantity of these substances produced is what is actually measured			
Immunological Methods	Serogroups of microorganisms based on the presence of different somatic antigenic determinants to differentiate E. Coli from various sources. Different serotypes of E. Coli are associated with different animal sources, although many serotypes are also shared among humans and animals.	--Can quickly discriminate human from animal sources (Scott, 2002).	--Requires a large bank of antisera (Scott, 2002).
Sterols or Fatty Acid Analysis	Sterols are constituents of the fatty acids in cell walls and membranes. This method differentiates between the types and quantities of sterols in human *E. coli* cell walls and membranes verses those in other animals. Fatty acids are first converted to fatty acid methyl esters (FAMEs) by chemical methods prior to performing gas chromatography.		--Under development with no published reports of use in fecal sourcing. --Requires access to Gas and/or HPLC chromatography equipment.
Antibiotic Resistance Analysis (ARA) **or Multiple Antibiotic Resistance (MAR)**	Uses fecal streptococcus (enterococci and/or *E. coli)* and patterns of antibiotic resistance to differentiate fecal pathogens. This method is based on human fecal bacteria having the greatest resistance to antibiotics and domestic and wildlife animal fecal bacteria having significantly less or different resistance to antibiotics. Most investigators are testing each isolate on 30 to 70+ antibiotic concentrations	--Less required training for lab personnel. --Lower per isolate cost (in time and materials). --Can perform the methods on hundreds	--Less precise. --Genetic instability or changes in antibiotic use can alter the resistance profiles obtained. (Carson, 2001). --Antibiotic resistance is often carried on plasmids that can be lost in cultivation or storage

Method	Description	Strengths	Weaknesses
Nutritional Patterns	Based on differences among bacteria in their use of a wide range of carbon and nitrogen sources for energy and growth. Another modification of the nutritional pattern concept is the use of human-specific (sorbitol fementing) bifidiobacteria as indicators of non-point source human fecal pollution.	--Method works well in a lab. --The BIOLOG system allows for the rapid performance, scoring, and tabulation of 96 carbon source utilization tests per isolate.	--Many environmental factors in a watershed that can affect bacterial nutrient requirements that may make this method impractical for field determination.
Chemical - Chemical methods do not detect fecal bacteria. These methods are designed to detect chemical compounds associated with humans. These chemicals are often found in wastewater such as septic tank effluent. Therefore, if the compound(s) are found in a water body then there is a human source.			
Coprostanol	Chemical indicator in human fecal pollution; it's a fecal stanol that is formed during catabolism of cholesterol by indigenous bacteria present in the gut of humans and higher animals and is the primary stanol detected in domestic wastewater.	--Some sterols have greater specificity for humans or animals (Scott 2002).	--Low sensitivity --Present naturally in sediments (Scott, 2002).
Optical Brighteners	Detects optical brighteners that are in all laundry detergents using low-tech black lights or mass spectroscopy. Samples are collected by placing optical brightener-free cotton in a wire mesh trap in a stream for a few days. Once removed, the cotton is examined with a black light to see if it glows. The fluorescent cotton can then be examined with mass spectroscopy to verify the presence of the compounds.	--If chemicals are detected, the source must be human.	--Persistent chemicals may not reflect recent pollution.
Caffeine Detection	In development - to find areas where humans are the source. Caffeine passes through the human digestive system and could be used as an indicator chemical.	--Useful for assessing impact from human sewage.	--Expensive - $100 per sample. --Plants with significant levels of caffeine could confuse results.

4.4 Ongoing Research - Pathogens, Antibiotics and EDCs

Analytical Methods

Title: Microbiological Impact of Agricultural and CAFO Activities on Surface Water Quality
Agency: USEPA ORD
End Date: September 2005
EPA is using bacterial source tracking in Turkey Creek Watershed, OK, to identify the source of fecal contamination in the watershed. The study uses antibiotic resistance analysis in combination with a statistical discriminant analysis to locate indicator organisms in the water, and then use a hollow-fiber ultra-filtration and real-time PCR technology to locate these specific microorganisms: human enteroviruses (poliovirus, coxsackievirus, echovirus), Yersinia enterocolitica, and Campylobacter jejuni. The results will help develop TMDLs and risk management strategies for land use practices in the animal industry (EPA ORD NRMRL).

Title: Methods for Detection of *Mycobacterium paratuberculosis* in Environmental Samples
Agency: USEPA ORD
End Date: August 2006
ORD is researching methods to better evaluate the presence of *Mycobacterium paratuberculosis* in water. A method utilizing polymerase chain reaction (PCR) is being developed to amplify the detection rate of this pathogen. It is believed that sample processing used with the PCR of a selected IS900 DNA sequence could be a suitable method for determining the presence of *M. paratuberculosis* until a better DNA sequence.

Title: Soil and Water Conservation Research
Agency: USDA CSREES
End Date: August 2005
Thurston-Enriquez, USDA ARS, is evaluating and adapting methods for characterizing human pathogens in, and dissemination from, animal manure and waste management systems. Recovery efficiency studies will be conducted for each sample type to determine the ability of each method to recover and detect the microorganisms under study.

Title: Environmental Behavior of Anthropogenic Chemicals Including Metal-Organic Interactions
Agency: USDA CSREES
End Date: September 2005
Lee, USDA CSREES, is identifying and quantifying reactions that control the persistence and distribution of contaminants in the soil and water environment, which directly influence their potential towards human and ecological exposure. A specific objective of this project is to identify the occurrence and quantify the environmental fate of major antibiotics and hormones commonly used in animal production in soil and water at feeding operations. Analytical techniques to be employed include gas and liquid chromatography, mass spectrometry, and radioassays.

Title: Fate and Transport of Sex Hormones From Poultry Litter Applied to Till and No-Till Cropping Systems
Agency: USDA ARS
End Date: September 2004
Jenkins, USDA ARS, conducted a field experiment to determine concentrations of the hormones estradiol and testosterone, and the pathogens *Salmonella* and *Campylobacter* in surface runoff and subsurface drainage from tilled and no-tilled soils to which fresh poultry litter is applied, and determine transport characteristics and degradation rates of estradiol and testosterone. Estradiol and testosterone were measured by competitive enzyme-linked immunosorbent assay. The pathogens were measured using enrichment and PCR most probable number methods.

Title: Rapid Detection of Agricultural Environmental Pathogens
Agency: USDA ARS
End Date: May 2004
Perdue, USDA ARS, utilized newly developed technology and instrumentation to develop and evaluate rapid molecular detection capabilities using real time PCR analysis of several agricultural pathogens.

Title: Advancing *Cryptosporidium parvum* Detection Methodologies
Agency: USDA ARS
End Date: March 2004
Jenkins, UDSA ARS is developing molecular and immunological techniques for detecting *Cryptosporidium parvum* in water. Fluorescence in situ hybridization (FISH), which utilizes DNA probes, has proven to be as effective as current detection techniques, while a method using continuous flow centrifugation was created to recover *C. parvum* oocytes from large amounts of water.

Title: Post-Harvest Survival Strategies and Biocontrol or Human Pathogens on Fresh Fruits and Vegetables
Agency: USDA ARS
End Date: May 2006
Bhagwat, USDA ARS is studying the effects of fresh-cut preparation practices have on produce and the survival strategies used by pathogens on the produce to develop post-harvest management practices that will limit pathogen grown. Green fluorescent protein (GFP) and gene array analysis will be used to monitor the adherence and reactions of the pathogens during the treatment processes.

Title: Development and Validation of Procedures for Monitoring Endocrine and Reproductive Function in Freshwater Mussels
Agency: USGS
End Date: Unknown
Gross, USGS is studying the effects xenobiotic agents have on the biology of freshwater invertebrates to determine the impact the agents have on their reproductive abilities. ecosystems. Radioimmunoassay (RIA) was used on tissue samples to analyze selected sex steroids and progesterone to determine any adverse effects.

Title: Mechanisms and Mitigation of Agrochemical impacts on Human and Environmental Health
Agency: CSREES IND
End Date: September 2005
Lee, CSREES is examining the effects of abiotic and biotic factors, in agricultural and natural environments, have on the reaction mechanisms, transformation rates, and fate of pharmaceuticals. Techniques including: Chromatograms, spectroscopy, radiolabel assays, and solvent-extraction techniques are used to examine changes in chemical concentrations and changes in the environments.

Title: Emerging Viral Diseases of Swine
Agency: USDA ARS
End Date: January 2007
Lager, USDA ARS, is studying swine influenza virus (SIV) virulence mechanisms utilizing a reverse genetics approach. They are developing and validating rapid diagnostic assays for the detection of (SIV) and evaluating an adenovirus-vectored SIV vaccine in young pigs.

Title: Emerging Zoonotic Diseases
Agency: USDA ARS
End Date: May 2006
Lager, USDA ARS, is studying zoonotic diseases in the United States (such as monkey pox). Many of these are foreign to the United States but can be easily introduced through movement of man and/or animals. Research goals include the development of a rapid diagnostic test, to determine the host range of monkey pox in domestic animals (those which man comes in contact with most frequently), and to develop a tactical plan to detect and control other incursions of emerging zoonotic diseases into the United States.

Title: Innovative Detection Methods and Improved Control of Ruminant Viral Pathogens
Agency: USDA ARS
End Date: January 2007
Ridpath, USDA ARS, is planning to improve industry's ability to detect and control viral infections of ruminants (principally cattle), with an emphasis on bovine viral diarrhea viruses (BVDV). Detection will be improved by the generation of robust field-ready tests that both detect and differentiate viral pathogens. Limiting and controlling viral infections will require research efforts to generate a more thorough understanding of viral/host interactions and identifying the mechanisms behind viral pathogenesis.

Title: Virus-Induced Respiratory and Reproductive Diseases of Swine
Agency: USDA ARS
End Date: November 2006
Wesley, USDA ARS, is determining the relative role of porcine reproductive and respiratory syndrome virus (PRRSV) and its interactions with other swine pathogens in the Porcine Respiratory Disease Complex (PRDC). They plan to identify mechanisms by which PRRSV establishes persistent infection in pigs, study the transmission of PRRSV, and study the genetic elements that contribute to virulence.

Fate and Transport

Title: Measuring and Modeling the Source, Transport, and Bioavailability of Phosphorus in Agricultural Watersheds
Agency: USEPA ORD
End Date: December 2005
ORD is studying the loss of Phosphorus to surface water in regards to the size of animal operations, manure management and crop production, the effect sediments have in the levels of P in surface water, and the fate and transport of P in surface water. This data will assist in the creation of improved models to monitor Phosphorus in agricultural watersheds.

Title: Pathogen Transport and Dissemination from Manure
Agency: USDA ARS
End Date: August 2005
Shelton, USDA ARS is attempting to develop realistic pathogen transportation models along the water pathways. EMSL created two kinematic wave models for surface and subsurface water flow were joined with a Green-Ampt infiltration model to simulate fecal coliform transport from manure application. The results were compared against the results of two sub-plots; it was determined that a one dimensional kinematic model can be used to predict fecal coliform concentrations within acceptable ranges.

Title: Integrated Management Regimens That Minimize the Environmental Impact of Livestock Manure
Agency: USDA ARS
End Date: April 2005
Cole, USDA ARS is attempting to determine the survival and transportation of pathogens from manures and feeding facilities, and the affects distiller grains has on animal performance and nutrient and pathogen excretion. There are no published findings yet.

Title: Detection, Survival, Transport, and Reduction of Human Pathogens From Animal Manure
Agency: USDA ARS
End Date: April 2005
Grieve, USDA ARS are evaluating the survivability and transport of pathogens located in manure and waste lagoons when transported in various forms. Soil column experiments indicate that the amount and distribution of manure particles in suspension can increase the transportation rates of pathogens.

Title: Model Abstraction Techniques for Soil Water Flow and Transport
Agency: USDA ARS
End Date: February 2005
Pachepsky, USDA ARS is researching the use of various modeling techniques to best simulate the movement of water and contaminants through soils.

Title: Manure Treatments and Uses to Protect Soil-Water-Air Quality, Food Safety, and Improve Manure Value
Agency: USDA ARS

End Date: April 2005
Sikora, USDA ARS is testing various methods for treating manures to reduce nutrient solubility, adjust pH, reduce emissions and pathogens, and improve the value of the final product. No findings have been published.

Title: Pathogenic Bacteria Breakthrough in Soils as Affected by Physical Meterogeneity
Agency: USDA ARS
End Date: August 2004
Pachepsky, USDA ARS is conducting soil column experiments to determine the transportation rates of bacteria through heterogenous soil samples. There are no published findings yet.

Title: Role of Soil Nematodes in Vectoring Pathogenic Bacteria to Fruits and Vegetables
Agency: USDA ARS
End Date: August 2004
Millner, USDA ARS is researching the attraction of nematodes to select pathogens, the effects the pathogens have on the life of the nematodes, the dispersal of the pathogens during and after the life of the nematodes, and efficacy of sanitizers on pathogens residing within the nematodes. Findings show that the studied nematodes were drawn to the selected pathogens, and that the nematodes released the pathogens up to five hours after removal from the source of the pathogens.

Title: Physical System and Tools to Study the Fate and Transport of Microorganisms in Porous Media
Agency: USDA ARS
End Date: July 2004
Van Genuchten, USDA ARS is using lysimeters to track the transport of microorganisms from feed lots to the air and groundwater in an attempt to find techniques to manage animal waste products to minimize pathogenic related disease and to create new methods for categorizing the fate and transport of pathogens. There are no published findings listed.

Title: Interrelations of Livestock Manures and Safety of Food Crops
Agency: USDA ARS
End Date: January 2005
Rice, USDA ARS is researching potential pathways by which pathogens and toxins are transferred to crops when manure or manure tainted water is applied, and if land treated with manure poses any hazards. A set of genetic markers was created for specific types of *E. coli* to track the transport and fate of the pathogen.

Title: Effluent Nutrient Contributing to the Survival of Microbes
Agency: USDA ARS
End Date: April 2005
Rowe, USDA ARS is studying 6 commercial swine lagoons to evaluate the environmental factors that contribute to the presence and fate of pathogens in irrigation water from the lagoons and the factors affecting their transfer into the soil. Experiments on soil troughs simulating a relatively slow drying process, post rain, show an elimination of human pathogens from the soil and manure.

Title: Detection and Fate of Microorganisms in Poultry and Swine Wastes
Agency: USDA ARS
End Date: January 2005
Rowe, USDA ARS is developing methods to profile the types and amount of pathogens in manure, soil, air, and plants in relation to the management systems used, environment, and time in an attempt to find out what chemical properties in the soil will affect the fate of the manure pathogens. The study has shown that managing the moisture level in poultry litter to control its rate of drying has resulted in the 99.9% destruction of the *Salmonella* pathogen.

Title: Safe Production of Vegetables using Manure
Agency: USDA ARS
End Date: September 2004
Millner, USDA ARS is researching methods of storing manure to reduce the number of pathogens, determining the survivability of pathogens on vegetable in growth chambers, and researching the composition of non-pesticidal control alternatives, "compost teas" that would limit the proliferation of pathogens. Results show that teas containing nutrient supplements allow pathogens to regrow, while teas without nutrient supplement containing compost prepared at high temperatures prevented *E. coli* and *Salmonella* from surviving.

Title: Pilot Study of Factors Affecting Maintenance of Mycobacterium, *Salmonella*, *E. coli* and *Listeria* on Dairy Farms
Agency: USDA ARS
End Date: August 2005
Perdue, USDA ARS is creating a pilot program of two dairy herds to generate a baseline of the pathogens that cause Johne's disease and food borne pathogens. The baseline will be used determine the best methods for managing animal health and product quality. No findings have been published.

Title: Elucidation/Environmental Parameters Controlling Vertical and Surface Transport of Pathogens
Agency: USDA ARS
End Date: April 2004
Shelton, USDA ARS is researching the relationship between vertical and surface movement in pathogens and the affects environmental factors, i.e., vegetation, rain, etc., on pathogen runoff to determine possible containment methods. Findings show that while bare test plots allowed 100% of the selected pathogen to be transported in runoff, only 1% of the selected pathogen cleared the vegetated test plot.

Title: Occurrence and Dissemination of Manure-Borne Zoonotic Pathogens
Agency: USDA ARS
End Date: August 2005
Thurston-Enriquez, USDA ARS is collecting water samples from feedlots and the surrounding areas, and bioaerosol samples to determine the concentrations and types of pathogens in the samples in an effort to determine and develop cheaper and more efficient manure management processes. The populations of manure-borne organisms released from fertilization is dependent on the type and age of the manure.

Title: Controlling the Growth of Human Pathogens on Fresh Fruits and Vegetables
Agency: USDA ARS
End Date: April 2006
Gross, USDA ARS is researching the biochemical and genetic mechanisms associated with the transport and fate of pathogens on fruits and vegetables. It is theorized that B-glucans assist *Salmonella* in adapting to life and reproduction on produce.

Title: Atmospheric Processes of Agricultural Pollutants that Affect Air and Water Quality
Agency: USDA ARS
End Date: April 2007
Hapeman, UDSA ARS is studying the interactions and affects agrochemicals, including veterinary pharmaceuticals, which are transported through the air, have on non soil or water surfaces they adhere to, and the ecosystems they enter. Water samples from rain falling through a forest canopy that came in contact with airborne agrochemicals were compared to water sample from rain falling just outside the canopy. The samples from inside the canopy almost always had higher levels of chemicals.

Title: Fate of Veterinary Antibiotics in manure Lagoons
Agency: USGS
End Date: February 2005
Kee Ong, USGS is researching the sorption and breakdown rates of the antibiotics tetracycline and sulfamethazine in manure lagoons with differentiating environments, i.e. pH, ammonia, salt levels. There are no listed results.

Title: Fate of representative Floroquinolone, Macrolide, Sulfonamide, and Tetracycline Antibiotics in Subsurface Environments
Agency: USGS
End Date: February 2005
Pedersen, USGS is researching the movement of selected antibiotics through soils containing varying types of natural organic matter. Observations will be made on the sorption rates of the antibiotics by the organic matter, and the potential of soils to act as antibiotic "sinks." There are no listed findings.

Title: Transport Behaviour of Sulfonamides and Tracers After Manure Application on Sloped Grassland
Agency: EAWAG (Swiss Federal Institute for Environmental Science and Technology)
End Date: Unknown
EAWAG conducted an irrigation experiment utilizing 12 grassland plots to measure the rates of runoff and vertical transport of a cocktail of liquid pig manure combined with sulfonamides and tracer elements against irrigation with deionized water. The amount of runoff was recorded as being four to six times higher on the plots irrigated with the manure mixture that with the water.

Title: Antibiotics in Livestock Production - Problems and Experimental Approach
Agency: EAWAG (Swiss Federal Institute for Environmental Science and Technology)
End Date: Unknown
EAWAG, is preparing an investigation to determine the amount of veterinary antibiotics used in Switzerland, how the excreted antibiotics move into the soil, and how long it takes for the

antibiotics to breakdown. In a series of two experiments, 12 plots will be fertilized and irrigated, the runoff and soil samples will be tested and the results compared against the transport data of known substances. A parcel of grassland will be fertilized and samples will be taken to determine how long the selected antibiotic will remain intact and how far it will travel. There are no listed results.

Title: Factors Controlling Veterinary Antibiotic Sorption to Soils
Agency: CSREES CONR
End Date: August 2005
Mackay, CSREES believes that a range of soil characteristics including clay, and oxides affect the ability of antibiotics to move through the soil, not just organic compounds. Thirty soil samples of varying profiles are being used to test the sorption rates of selected antibiotics. No results yet.

Title: Examination of Estrogenic Chemical Behavior for Determining Potential Off-Field Migration from Dairy Manure to Texas Waters
Agency: Texas A&M Univ.
End Date: Unknown
Kenimer, TAMU is planning a study to identify the concentrations of select estrogen compounds in dairy waste lagoons, what the metabolites of the compounds are, and determine the sorption rates and characteristics of the estrogen compounds and metabolites in the soils receiving manures and wastewater. There are no listed results.

Strength of Evidence

Title: Preventing Pathogen Transport to Southern Piedmont Landscapes from Poultry Production Systems
Agency: USDA ARS
End Date: April 2005
Jenkins, USDA ARS is developing a baseline assessment of pathogens in the Southern Piedmont watershed where high levels of poultry production occur and methods for source identification. DNA fingerprinting is feasible for his study, but requires the development of an extensive library of fingerprints that may not be economically feasible at this time.

Title: Development of a Quantitative Detection Method for Enumerating Host-Specific Fecal Bacteria Based on Real-Time, Quantitative Polymerase Chain Reaction
Agency: USGS Water Resource Grant
End Date: February 2005
Nelson, USGS is developing and evaluating a quantitative method for calculating the fractional contribution of fecal pollution from humans and animals using real-time, quantitative PCR based on human and cow-specific nucleic acid sequences. This method is designed to be used independently or with other MST techniques.

Title: Microbial and Animal Source Tracking in Mississippi Waters
Agency: The University of Southern Mississippi
End Date: Unknown

Researchers are conducting MST research using molecular methods (antibiotic resistance analysis, PFGE, and rep/BOXPCR) to fingerprint isolates. They have determined that several techniques are useful in MST and are developing a database for *E. coli* and *Enterococcus* sp. from the southern and coastal portions of Mississippi. The database is available online.

Title: Microbial Source Tracking (MST) in the Soil Microbiology Laboratory at the University of Georgia
Agency: University of Georgia
End Date: Unknown
Hartel, UGA is examining the use of ribotyping and ratios of various enterococcal species to determine sources of fecal contamination from specific animal species. He is also testing other bacteria with limited-host ranges.

Title: Bacterial Source Tracking (BST)
Agency: Virginia Polytechnic Institute
End Date: Unknown
Hagedorn developed a database of over 4,000 fecal streptococcus isolates (human, cattle, sheep, horse, deer, and geese) from antibiotic resistance profiles. Using the antibiotic resistance profiles, they differentiated between human and animal isolates and reduced fecal coliforms in Page Brook by an average of 84% between 1997 and 1999.

Title: Identifying Sources of Fecal Coliform Bacteria in Selected Streams on Virginia's TMDL Priority List
Agency: Virginia USGS
End Date: Unknown
Hyer is determining the effectiveness of MST (ribotyping for *E. coli*) to identify the sources of fecal contamination in stream segments covering a range of ag and urban sources, and evaluating the utility of source tracking to enhance model waste-load allocations, calibration, and verification.

Title: Development of Bacterial Source Tracking (BST) Libraries and Assessment of Bacterial Sources Impacting Lakes Waco and Belton
Agency: Texas A&M University Agricultural Experiment Station
End Date: Unknown
Di Giovanni is developing a publicly available library of genetic fingerprint and antibiotic resistance profiles generated from known animal, human, and wastewater *E. coli* isolates from the Bosque and Leon River watersheds. Future studies using genotypic and phenotypic BST methods will expand libraries in order to exchange information with other researchers and regulatory agencies. This study uses EPA Clean Water Act 319 funding.

Title: Presence and Survival of Fecal Indicator Bacteria in Soil from the Banks of Major Rivers and Streams on Guam
Agency: USGS Water Resources Grant
End Date: February 2005
Denton is testing whether *E. coli* and enterococci are capable of surviving in Guam riverbank soils over extended periods of time, and discovering important soil-related factors. This research

will help determine whether indicator bacteria can survive tropical and subtropical soils and whether alternate indicator organisms need to be used in warm climates.

Title: Application of Innovative Methods and Strategies to Differentiate Sewage from Non-Point Source Pollution in Hawaii
Agency: USGS Water Resources Grant
End Date: February 2005
Fujioka is assessing the reliability of monitoring Hawaiian waters for *C. Perfringens* and another alternative fecal indicator for the purpose of developing the best combination of test methods and strategy to reliably determine when a warm-climate environment is contaminated with sewage or non-human contamination.

4.5 Summary of Pharmaceuticals and Pathogens

Pharmaceuticals and Pathogens from AFOs may have a negative impact on human health and the environment. Reviewed research provides information on the:

- analytical methods that test for veterinary pharmaceuticals and microorganisms found in the environment and are linked to adverse human health effects;
- fate, transport, and environmental impacts of pharmaceuticals and pathogens; and
- linkages between outbreaks of disease and pathogenic organisms.

Research is currently focused on a limited number of areas of pathogen and veterinary pharmaceutical fate, transport, and environmental impacts. Research needs to occur for the broad range of organisms and chemicals. Adaptive reuse of existing models could facilitate in the development of pertinent models. Standardization of analytical methods will allow for quality assurance and quality control procedures to be valid. In addition, a national database or repository of models, methodologies, methods of transport, or genotypes (for genetic testing), etc. could reduce duplication of work. As more studies are conducted, the evidence for linking AFOs to human and environmental affects may become stronger. This information will allow for the best possible resources and the most streamlined approach for determining the source of AFO contamination.

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